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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

OBSERVATIONS ON ENDAMEBIC DYSENTERY*

BY HARRY S. HUTCHISON, M.D.,† TANTA, EGYPT

SINCE Losch discovered amebas in a patient suffering from chronic dysentery in 1875, much has been done to increase our knowledge of this organism and its relation to the pathologic process with which it is associated in the human body. Its study in relation to the production of disease was much confused, because at first, it was not recognized that there are several distinct species of amebas living as parasites in the human colon, only one of which causes a pathologic process leading to clinical disease. The further fact that the pathogenic species may exist in the colon of its host for a long time without producing clinical evidence of its parasitic action, has been clearly demonstrated by Walker and others and does much to explain the difficulties connected with Endameba histolytica infection. These facts must be borne in mind by anyone who seeks to solve the problem of etiology of dysentery either clinically or in the laboratory.

It is a common experience in subtropical climates to find two people harboring the same parasite, the one having severe clinical disease and the other remaining symptomless. In the courses of routine examinations of the stools of English and American foreigners in Tanta Hospital, Endameba histolytica cysts are not infrequently encountered where the person has had no previous history of dysentery. Warthin, Wenyon, and O'Connor, Kofoid, and Mathews, have shown how common were infections with Endameba histolytica among men who lived under the none too hygienic conditions during the war. Numerous observers are calling the attention of medical men to the infrequent cases of amebiasis occurring in our temperate climate.

The pathologist is often the court of last appeal in settling the question of amebiasis, and it seems worth while to consider it from his standpoint. Much has been written on the pathology of amebic dysentery, and it has

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been well done. The descriptions of Councilman and Lafleur, written in 1891, remain the classic on the subject. The facts laid down by them have been further amplified by Dopter, Kuenen, Christopherson, and recently by Callender. There are, however, facts that bear repetition and seem particularly worth emphasizing after experience in the tropical climate of Egypt where amebiasis is common, and in America where an understanding of the disease is uncommon.

In the American Hospital at Tanta, which cares for both foreign and native patients, 2.7 per cent of the patients treated in the past three years have had clinical amebic dysentery. This does not represent the number of patients harboring the parasite. The incidence of amebiasis, by which is meant demonstrable infection with Endameba histolytica, is very high among the native people. Adequate surveys to show just how high have not been made, but the incidence is certainly so high as to be almost universal among the native population.

The small number of patients suffering from definite disease due to this infection with Endameba histolytica is only explainable by a clear conception of the life history of the parasite and the factors concerned in its invasion of the host. The ideal condition for the parasite is a nice adaptation to the host so that there is no disturbance of function which will bring the defense mechanism of the host into play against the parasite. The accepted story of the life of the Endameba histolytica shows how this can be. The normal habitat of Endameba histolytica is the human colon. The active part of its life cycle is entirely confined here. Dissemination to other hosts occurs in a resistant inactive form, the cyst. The active vegetative organism loses its activity, decreases in size, and forms about itself a resistant capsule. In the encapsulated state, the nucleus divides and subdivides to form four smaller nuclei. In mature cysts there are always four nuclei and very seldom, probably never, more. (Dobell.)

The cysts are found in the normal or nearly normal stools of persons who have recovered from dysentery or who may never have had any symptoms. The cysts are easily injured by drying, heat, cold, or sunlight. Conditions in Egypt and other tropical and subtropical countries are such that transfer of cysts to fresh food materials is common. Chief among the agencies of transfer are hands, water, and flies.

An illustration or two will make this clear. The Mohammedan is required by religious law to wash himself after defecation, not only externally but also the lower portion of the rectum. Because of this fact, the bank of a stream or canal is the most convenient latrine. Water in the canals where there is a dense population is commonly grossly contaminated. Since the washing is performed with the bare hand and fingers, the nails especially are grossly contaminated with the persons's own feces. Those who are so contaminated and act as food merchants or cooks are constant sources of food contamination.

In tropical and subtropical countries, the water is taken directly from the canals for irrigation and for use in the homes. Fresh vegetables which may

be eaten uncooked are fertilized with night soil, irrigated with canal water, and finally kept fresh in market by dipping in canal water.

Flies abound in all climates, especially in the tropics, and where fecal matter containing cysts is exposed, they feed upon it. It has been proved that the cysts of Endameba histolytica survive in the intestines of the fly and pass through it unchanged. (Wenyon and O'Connor, 1914-1916.) If the cysts are deposited by the fly on human food, they readily find access to the gastro-intestinal tract. It has been proved that only mature cysts are infective when eaten. (Walker and Sellards.) Under conditions existing in the small intestine, the cyst wall ruptures and a living ameba escapes. It is generally believed that one organism containing all four nuclei emerges and that this then divides to form four new amebas. No sexual phase has been demonstrated.

As soon as the young parasite comes in contact with the mucosa of the large intestine, it attaches itself to the lining epithelial cells. Probably by a process of cytolysis a superficial erosion of cells is produced, and the parasite lives in and upon the dissolved cells, penetrating downward as the process of cytolysis continues and living in the cytolized material close to the living cells. As the parasite invades new tissues, the host endeavors to limit and repair the damage done. Usually a state of equilibrium is reached early and the ulceration never becomes extensive. If the lesions are very mild, no subjective or objective symptoms may be noted, and the individual becomes a contact carrier. The cysts from his stool are fully virulent and capable of producing severe clinical disease in a suitable host.

The initial infection often leads to a slightly more extensive ulceration of the intestine, and the symptoms of diarrhea or dysentery supervene. The attack is quickly overcome and the person becomes symptom-free. There are still, however, cysts in the stool, and there may be more or less frequent return of symptoms, depending on such things as indiscretion in diet, work, exercise, or exposure. This condition is known as the convalescent carrier. (Walker and Sellards.) The contact carrier condition is frequently encountered among the native people of Egypt and is seen among foreigners. The convalescent carrier condition is the common condition among the latter.

When a condition of equilibrium between host and parasite has been nearly reached and dysenteric symptoms have abated, some of the amebas in the tissues become inactive, lose by egestion the engulfed red cells and other food in the cytoplasm, decrease in size, and are discharged from the ulcers into the lumen of the gut. This is the precystic form. A resistant capsule is formed about the parasite as it continues to diminish in size; its nucleus divides and redivides to form four smaller nuclei; chromatin bodies may form in its cytoplasm, and it is now known as a cyst. In the cystic form the parasite passes from the colon of its host, and the cycle is completed.

Evidence shows that if the parasite passes from the colon of its host before the cysts are fully formed, or in the vegetative condition, they quickly perish and therefore cannot infect a new host. (Walker and Sellards.) It is only when the fecal stream through the bowel is nearly or altogether nor-

mally prolonged that there is time for the formation of cysts. In kittens where a severe fatal dysentery is the result of infection, cysts do not appear, and in the liver, lungs, and other tissues, cysts are not found.

If these facts are kept in mind, it will be seen why the search is for cysts or precystic forms in the normal or nearly normal formed stool, and for amebas in the dysenteric stool and in the tissues.

It is not within the scope of this paper to discuss the differential characteristics of the vegetative, precystic, and cystic forms of amebas. The reader is referred to the abundant literature on this subject.

Apparently all strains of Endameba histolytica are equally virulent, but among a number of persons exposed to a given source of infection, a comparatively small number will have clinical evidence of the infection, and the remainder will show only slight signs or none at all. It is a common experience in the tropics to find people showing no symptoms, yet having cysts in their stools. In postmortem work where the possibility of mild to insignificant lesions is kept in mind, a considerable number of amebic ulcerations are discovered in the colon from patients who have died from other causes. Reviews of seven cases that occurred in Pittsburgh and Cleveland showed all to be very severe with overwhelming lesions. Failure to discover milder infections, in the light of experience with the disease in the tropics, suggests the possibility that many mild to insignificant amebic lesions are overlooked in our northern hospitals. Certainly this is true clinically, for of the seven cases all of which were severe and four of which were complicated by liver abscesses, only two were diagnosed clinically and one other suspected.

Where there is an infection in the colon, the ulceration may extend as a superficial erosion or as a deep penetration of the tissue. The former exists very early and is limited in extent. The latter is the common and more typical lesion. The cytolytic activity of the parasite results in a deep, well-like necrosis of the mucosa, and the parasites migrate down through the necrotic material and lie in close proximity to the living cells below. The muscular coats, first the muscularis mucosa and later the muscular wall of the colon, offer resistance to the progress of the lesion, and the ulceration spreads laterally under the mucosa, forming small localized lesions characterized by a narrow perforation of the mucosa communicating with a crater in the submucosa. This is the "bouton de Chemise" lesion of the French writers. Grossly, it looks like a yellow, necrotic, slightly raised point, ranging from a pinhead to a pea in size, and located in the mucosa of the colon. Such a lesion might be mistaken at a casual glance for a caseous tuberculous nodule. On section, it shows a narrow orifice, undermining crater, a central more or less necrotic jelly-like mass, very slight surrounding inflammatory reaction, and amebas. This lesion may be the only one present to gross inspection, or it may be, and often is, seen in connection with more advanced lesions in the severely ulcerated colon. As the lesion extends, it increases in size and may penetrate the muscular coats. The amebas advance with the necrosis and remain in large numbers near or sometimes penetrating the cells of the living tissues. If the ulceration is progressive, several amebas may be found in the blood vessels and lymphatics supplying the involved area of

gut. Secondary bacterial infection soon occurs from contact of the ulcers with the fecal stream. The injured cells become more definitely necrotic, inflammatory reaction sets in, and the clinical picture of dysentery is present. The undermined mucosa is cut off from its nutrition, dies, and is cast off as sloughs, leaving ragged ulcers with overhanging edges, necrotic bases, and surrounding inflammatory reaction. As the ulcers enlarge, they become confluent, first by the formation of submucosal tunnels, and later from the death of the overlying mucosa, by channels of ulceration. The intestinal mucosa is pitted and grooved in various sized and shaped areas of ulceration, separated by islands and isthmuses of mucosa. The ulcers may penetrate the muscular coats of the intestines and the serosa, allowing the contents of the gut to escape into the abdominal cavity. As a rule, however, the process is not so rapid, and inflammatory reaction about the ulcerated area produces thickening of the gut wall by fibrous tissue and adhesions to surrounding tissues. The bladder and genital tract have been invaded by extension from an intestinal focus.

The ulceration may spread so rapidly in the intestinal mucosa as to cause the overhanging mucosa to fall away from the submucosa before it has had time to become necrotic. Hemorrhages occur from eroded vessels, and the blood forms cobweb-like clots in the ulcers, which give the gut a shaggy appearance. Such was the appearance of the colon of an elderly man who went on a world tour for the sake of his health, contracted amebic infection in the Orient, and died in Pittsburgh this winter. It represents what may be expected in amebic infection when the reparative powers of the host are at a low level. In another patient, in Pittsburgh, whose resistance was lowered, 24 centimeters of the colon were completely gangrenous. Still another case of very severe ulceration and gangrene occurred in a native Egyptian, who was brought to the hospital because of a fracture of the spine in the upper dorsal region. At the time of admission there were no dysenteric symptoms. There was complete paralysis of the voluntary muscles on both sides below the level of the fractured vertebra, and the patient rapidly developed a most dramatic amebic dysentery and died with a gangrenous intestine. Just what part is due to the amebic infection and what to secondary invaders in cases like these is impossible to say. Certainly the widespread destruction would not have occurred without amebas.

The earliest lesions of amebiasis are found in those portions of the colon where stasis is most likely to occur. Cecum, rectum, sigmoid, colon, splenic flexure, and hepatic flexure show ulceration in diminishing frequency as named (Clarke). In severe cases the colon is likely to be involved throughout its length. The appendix is commonly involved in cases where there are extensive lesions in the cecum. Amebic lesions of the appendix were not encountered in a review of 24 appendices (13 from natives), which were brought from Egypt.

In the primary lesion of the collar-button type, with a jelly-like necrotic slough, amebas are easy to find. Such lesions may be found alone or in conjunction with others, and are to be sought and sectioned. When the ulcers become infected with bacteria, amebas are found less numerously. They are

found in the deeper parts of the necrotic base in close proximity to the living tissue, or still more often in areas of necrosis under the overhanging edges of ulcers. Amebas may be seen penetrating among the living tissue cells, in the margins of the ulcers or in small blood vessels near them. They occur in groups, are discrete from the tissue cells, and are usually surrounded by a clear zone. The more rapid the process of ulceration and the more violent the dysentery, the more numerous the parasites. Fewer and fewer parasites are seen in lesions as the ulcers become less progressive, and healing becomes evident. Neither in the sections from Egypt nor in cases in America have amebas been found in the presence of granulations. They may be found in ulcers that show healing, but there is no healing in the portion of the ulcers where the amebas are found. The colon of a nurse who had amebic dysentery (diagnosed in Pittsburgh and treated vigorously with emetin before death) showed very extensive ulcerations of the type expected in chronic amebiasis, with fibrous thickening of the gut wall. Grossly there was much evidence of epithelialization of the ulcerated areas, and microscopically there was much granulation tissue. Many sections were made but no definite amebas were found.

Often extensive ulcers may show few amebas after secondary bacterial invasion has occurred and the walls of the intestines have become filled with inflammatory exudate. This may be because the ulcers started by the amebas have been spread more rapidly by the secondary invaders, and the inflammatory reaction exerts an unfavorable influence on the amebas. It is certain that the extent of the ulceration appears at times to be out of all proportion to the number of amebas present, and careful search is necessary to bring them to light.

It has been pointed out (Callender) that the type of ulceration seen in an intestine or the type of cells in a stool are not always trustworthy guides in the diagnosis of dysentery. Bacillary dysentery may coexist with amebic. The bacillary picture may be superimposed on the amebic picture. Conversely the finding of amebas in an ulcerated gut does not necessarily prove that the clinical manifestations of disease are due to its presence. Neither does the typical superficial ulceration of bacillary dysentery and the recovery of an organism mean that search for masked ulcers of amebic origin is unnecessary. Similarly the finding of vegetative or encysted amebas in a stool does not necessarily mean that the patient suffers from amebic dysentery.

The character of the stool in amebiasis depends very greatly on the extent of the ulceration, the position of the ulceration high or low in the colon, and upon the type of secondary bacterial invaders present. As a rule, the stool is foul, mixed with feces and swarming with organisms. It is likely to be small in amount in severer cases and may be largely mucus. As the condition of the patient improves, the stool becomes more fecal in character, of a pea-soup consistency, brown in color and containing considerable quantities of blood and mucus. The mucus is found as small masses of sago-like material stained with blood and floating in the liquid feces. In very acute cases showing such stools, a loop full of feces is always searched first for motile vegetative amebas. It is spread on a glass slide and pressed out

under a cover slip and examined at once. The more acute the ulceration in the bowel, and therefore, the more acute the disease, the shorter is the time that elapses between leaving the host's tissue and examination; consequently the better the chance of finding the organism motile. If the ulcers are healing, or partially so, and the host is resisting his infection, the diarrhea will be less violent; the chances for the organism to begin the process of encystation will be greater, and many of the amebas will become inactive, and even though kept under ideal conditions on a warm stage it may be difficult to detect motility. Only motile, active amebas ingest red blood cells, and so this diagnostic sign should not be expected under conditions which produce precystic and cystic forms.

In diarrheic stools or formed normal stools, such as are obtained from convalescent carriers with a small amount of ulceration in the intestine, many precystic forms and cysts will be found in the stool. If the stool has on its surface a trace of blood or a bit of bloody mucus, this should be especially searched. The specimen is macerated with a drop of normal saline on a glass slide and examined unstained with the low power objective against a dark background. Amebas, cysts, and precystic forms appear as bright, rounded, translucent bodies of uniform or irregular size, depending on whether they are in the same stage of development. No time is wasted looking for motility unless the condition of the patient and character of the stool would indicate that the disease is acute or very active.

To bring out the cysts and make it possible to count their nuclei, the fecal suspension is mixed with a watery solution of iodine in potassium iodide "the stronger the better." (Dobell.) A drop of strong tincture of iodine may be used if it is put on the slide first and allowed to evaporate almost to dryness. The drop of emulsion of feces is then rubbed up in the center of the iodine area.

To examine the nuclear structure of amebas and cysts, fixation of perfectly fresh specimens while they are still wet is necessary (Dobell, 1919).

Amebic dysentery is an unfortunate event from the ameba's point of view. The organisms are forced into the outer world out of their normal habitat so suddenly that they have no time to encyst themselves. Just as unfortunate from the point of view of the parasite is the formation of secondary lesions in other parts of the host than the colon, because here again cyst formation does not occur and there is no possibility of completing the life cycle.

The most common secondary lesion is liver abscess. The intestinal ulcers furnish the atrium of infection and amebas pass by way of the blood stream to the liver. The first evidence of their presence in the liver is a hepatitis; then small abscesses form. Probably, at first, they are multiple, and although usually in the right lobe, they may occur in any part of the liver. The small early lesions contain a central, nonpurulent, necrotic mass and many amebas. The abscesses increase in size due to the action of the parasites and coalesce to form larger abscesses. It is not until the abscess reaches a considerable size that it can be diagnosed clinically. This may be the reason that some authors have described liver abscesses as usually solitary. Experience has

taught that in many cases they are multiple. As the size of the necrotic area increases, the abscess content is likely to be invaded by bacteria which change its character, giving it a purulent nature. Rupture of blood vessels in the necrotic walls allows the escape of blood into the cavity and imparts to the mixture the characteristic anchovy sauce appearance.

The character of the wall of the abscess depends somewhat upon the course and duration of the lesion. If the abscess is recently formed, the wall will consist of a necrotic zone of tissue gradually passing over into living tissue cells. In abscesses of purely amebic origin there is necrosis of tissue without pus formation. The amount of inflammatory reaction in the wall depends on the character and presence of secondary invaders. If the progress has been slow or kept at a standstill by treatment or by the resistance of the patient, there will be plentiful fibrosis in the abscess wall.

In amebic abscesses that are increasing in size, the wall is filled with smaller secondary abscesses which contain an abundance of amebas. As the smaller abscesses increase in size, they coalesce with the main abscess and become part of it. The result is that the inside of the amebic abscess has an irregular sloughing wall with many larger and smaller cavities opening into it. The microscope shows multiple small early and larger later abscesses in the zone about the main abscess. Amebas are found in all abscesses but are most numerous in the smaller ones and always near the living tissue.

In old, long standing, secondarily infected and treated abscesses, amebas may be hard to find. When they are few, great care must be exercised not to confuse large tissue cells occurring singly with amebas. The ameba does not normally contain fat, so fat stains help to differentiate them from the large vesicular fat-bearing cells that are sometimes seen packing the spaces between fibrous tissue bands and penetrating among stroma cells near chronic abscesses. Sections carefully stained with iron hematoxylin after fixation in bichloride solution bring out the differential characters of the nuclei (Dobell, 1919).

The lung may be involved secondarily to abscess of the liver by perforation of the diaphragm and pleura. There is no essential difference between the progress of the abscess in the lung and in the liver.

Abscess in the brain arises, as a rule, from the excitation of operative procedures on abscesses of the liver. It is undoubtedly embolic in origin. No cases of brain lesion have been encountered in this study.

Amebic lesions occur in other regions of the body. They are nearly, if not always, due to extension of a colon lesion to a devitalized area. Infection of the bladder, testes, and seminal vesicles, peritoneum, abdominal wall, and skin have been discovered, but such lesions must be regarded as more or less accidental. Smith records an amebic infection of the knee joint with multiple sinuses. Kofoid and his associates, by a method of their own, claim to have demonstrated amebas in the lesions of chronic arthritis and in the lymph nodes of Hodgkin's disease. Their results have not been generally accepted.

CONCLUSIONS

1. The correct interpretation of an amebic lesion depends upon a clear recognition of the status which exists between the host and parasite, and the secondary modifying factors which may intervene to obscure the picture.

2. The fact that amebic infection exists without symptoms or with very minor symptoms and minor lesions often leads to failure in recognizing cases.

3. Recognition of cases without symptoms is possible only through search for cysts in the stool. Experience has taught that even then only positive findings are conclusive, as cysts may not appear in the stool of carriers over considerable periods of time, depending upon the extent of the lesion and whether or not it is progressing or regressing.

4. Recognition of cases with dysenteric symptoms consists in the identification of vegetative amebas, precystic forms or cysts in the absence of more obvious etiology.

5. The recognition of amebic ulceration depends upon the gross appearance only as it is suggestive; the final decision rests on finding amebas in the lesions.

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STUDIES OF THE ACTION OF SODIUM THIOSULPHATE IN METALLIC INTOXICATIONS*

I. The Effect of Sodium Thiosulphate on Arsenic Elimination

By A. G. Young, Ph.D., Ann Arbor, Mich.

IN 1920 Ravaut[1] reported beneficial results obtained by treating arsenical dermatitis by intravenous injections of sodium thiosulphate. This was followed by the work of McBride and Dennie[2] in this country. Since then the use of this compound has been extended to the treatment of other metallic intoxications, such as those due to lead, bismuth, mercury, etc.[3] The work so far consists largely of clinical reports. The general opinion at first seemed to be that sodium thiosulphate increased the rate of elimination of the toxic substance, thereby exerting its beneficial effect. Groehl and Myers[4] and Myers, Marples, Groehl, and Throne[10] report that sodium thiosulphate increased the rate of arsenic excretion in metallic toxemias. Kuhn and Loevenhart[5] experimenting on rabbits found that sodium thiosulphate reduced the total amount of arsenic excreted per twenty-four hours. Later Kuhn and Reese[6] reporting on clinical cases said that: "The treatment increases the excretion of arsenic and hastens the restoration of the kidneys to normal."

Table I

Showing the Effect of Sodium Thiosulphate on Arsenic Tolerance

CONTROLS			TREATED			
	NA-ARSENATE MG. PER KG.	LIVED		NA-ARSENATE MG. PER KG.	NA-THIOSULPHATE MG. PER KG.	LIVED
Rabbit No. 13			Rab. No. 10			
4/23/25	25		4/ 7/25	30	50	
4/29/25	15		4/17/25	30	—	
5/ 5/25 Dead		12 days	4/23/25	30	—	
Rab. No. 15			4/24/25	Dead		17 days
4/23/25	25		Rab. No. 16			
4/29/25	15		4/23/25	25	—	
5/ 5/25 Dead		12 days	4/24/25		50	
Rab. No. 1			4/29/25	15	—	
3/27/25	25		5/ 8/25	15	—	
4/ 1/25	25		5/12/25		50	
4/ 3/25 Dead		8 days	5/15/25	15	—	
			5/16/25	—	50	36 days
			Rab. No. 17			
			4/24/25	25	50	
			4/29/25	15	—	
			5/ 8/25	15	—	
			5/12/25	—	50	
			5/15/25	15	—	
			5/16/25	Killed		32 days

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These reports are conflicting and suggest the following questions:

a. Does sodium thiosulphate act differently in man than in rabbits?

b. Does it act the same on organic as on inorganic arsenic?

The work herein reported was undertaken in an attempt to answer the above questions.

METHOD

Rabbits.—Sodium arsenate was administered intravenously to rabbits in doses of from 25 to 30 mg. per kg. of body weight. Half of each group of rabbits received sodium thiosulphate intravenously in doses of 50 mg. per kg. at intervals between the arsenic injections, as shown in Table I. If the animal survived the initial dose of arsenic, a second dose of 15 mg. per kg. was given. At the time of death the animals were autopsied and examined for gross lesions.

Results.—Table I shows the amount of arsenic and thiosulphate given, and the number of days the animals survived. Two of the animals receiving no thiosulphate succumbed on the twelfth day after receiving but 40 mg. per kg. of sodium arsenate in two doses of 25 and 15 mg. respectively. The third one survived but two days following a second injection of 25 mg. per kg. of sodium arsenate. The three animals receiving thiosulphate treatment lived seventeen, thirty-six, and thirty-two days respectively, one requiring three doses each of 30 mg. per kg. to produce death, another required one dose of 25 mg. and three doses of 15 mg. per kg. to produce death. Rabbit No. 17 survived one dose of 25 mg. and three doses of 15 mg. per kilo but had to be killed at the end of the thirty-second day because of an injury. It will be noticed that rabbit No. 10 survived seventeen days after receiving but one injection of thiosulphate.

TABLE II

SHOWING THE EFFECT OF SODIUM THIOSULPHATE ON ARSENIC EXCRETION IN RABBITS

RABBIT	MG. NA-ARSENATE PER KG.	TOTAL AMT. ARSENIC GIVEN, MG.	URINE ANALYSIS MG. AS. EXCRETED			MG. PER KG. NA-THIOSULPHATE
			24 HR.	72 HR.	PER CENT TOTAL AS. GIVEN	
1.	25	28.38	No urine	2.1 mg.	7.4	None
5.	30	27.46	4.42 mg.	No urine	16.1	None
6.	30	30.09	4.08 mg.	No urine	13.2	None
7.	30	34.52	4.35 mg.	No urine	12.6	None
9.	30	17.59	2.21 mg.	No urine	12.6	None
					Av. Excr. =	12.38%
2.	25	29.96	No urine	2.51	8.4	50
4.	25	41.69	3.21	No urine	7.7	50
8.	30	20.19	2.08	No urine	10.3	50
10.	30	35.39	2.43	No urine	6.3	50
					Av. Excr. =	8.17%

This data shows that the thiosulphate increased the rabbits' tolerance to repeated sublethal injections of sodium arsenate. It is therefore interesting to note that it would not prolong the animal's life if single large doses of the arsenical were given. That is, it does not increase the M.L.D. to a marked extent as shown by the following data:

The M.L.D. of sodium arsenate for rabbits is 45 mg. per kg.[7] This dose was administered to a group of five rabbits. The injection was followed im-

mediately by an injection of 50 mg. per kg. of sodium thiosulphate. Four of the five animals survived while without the thiosulphate it is safe to predict that three or more would have died. A second group of five rabbits was given 50 mg. per kg. of sodium arsenate followed by the same amount of

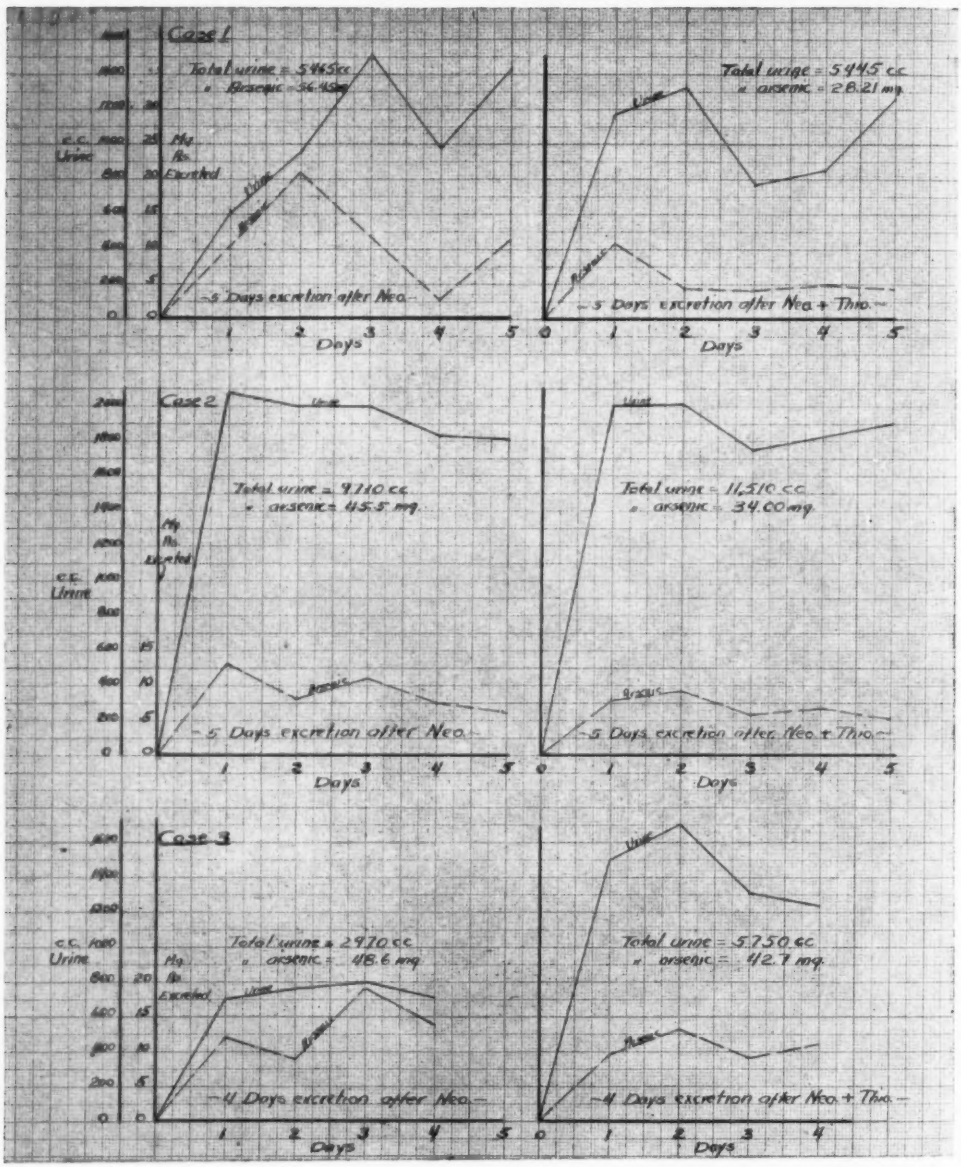
Case 1
Total urine = 5465 cc
" Arsenic = 36.45 mg
c.c. Urine
Mg. As. Excreted
Urine
Arsenic
5 Days excretion after Neo.
Days
Total urine = 5445 cc
" arsenic = 28.21 mg
5 Days excretion after Neo. + Thio.
Case 2
Total urine = 9710 cc
" arsenic = 45.5 mg.
Total urine = 11,510 cc
" arsenic = 34.00 mg.
Case 3
Total urine = 2970 cc
" arsenic = 48.6 mg.
Total urine = 5750 cc
" arsenic = 42.7 mg.
4 Days excretion after Neo.
4 Days excretion after Neo. + Thio.

Chart I.

sodium thiosulphate. Of this group only one survived. This shows that the thiosulphate was not a protection against an approximate increase of 10 per cent of the M.L.D. The same results are reported by Voegtlein using sodium arsenite and arsenoxide.[9] Table II shows the effect of sodium thiosulphate on the arsenic excretion of rabbits. Two groups of animals were used. The

first group received no sodium thiosulphate while the animals of the second group received 50 mg. per kg. of sodium thiosulphate following the administration of the arsenic. Examination of this table shows that the average arsenic excretion of the animals receiving thiosulphate was 35 per cent lower than the average excretion of the animals receiving no thiosulphate.

This confirms the finding of Kuhn and Loevenhart[5] who reported that sodium thiosulphate reduced the total amount of arsenic excreted by rabbits per twenty-four hours.

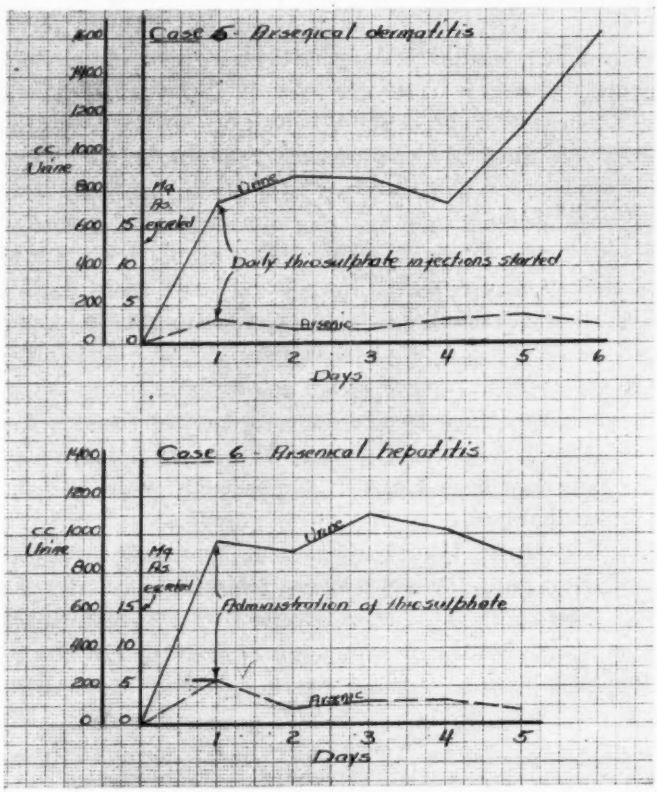

Chart II.

CLINICAL INVESTIGATION

In reviewing the clinical literature to date one finds many case reports, but few of them are accompanied with quantitative studies of arsenic excretion before and after the thiosulphate was given. In the references quoted above[4, 6] one finds that a twenty-four-hour specimen of urine taken previous to the administration of the thiosulphate has been considered indicative of the rate of excretion of the arsenic. Thiosulphate was then given and the urine analyzed for five or six days in succession.

Since the quantity of urine voided from day to day varies considerably with the diet, fluid intake, etc., it seemed logical to expect that the arsenic output in cases of intoxication might vary from day to day also. In two cases of arsenical intoxication which I have studied the administration of thio-

sulphate was followed by a decrease in the arsenic excretion as determined on twenty-four-hour samples taken before and after its administration. However, since the doubt existed as to the value of such findings the following method was used: Patients receiving arsphenamine or neoarsphenamine but who showed no toxic symptoms were selected after they had received four or five weekly treatments. Following a single treatment, twenty-four-hour specimens of urine were collected for four or five days. Each sample was analyzed to determine the amount of arsenic excreted each day under normal conditions and also to determine the variation in the rate of elimination from day to day. The following week when the patient received the arsphenamine treatment, sodium thiosulphate was given intravenously for four or five days in daily doses of 10 c.c. of a 10 per cent solution. The urine was collected as before and analyzed. Four cases were studied. Chart I shows the results obtained in three of the cases studied in this way. Examination of this chart shows that in Case 1, approximately the same volume of urine was excreted during each five-day period but that only one-half the amount of arsenic was excreted when the sodium thiosulphate was given. It also shows that the amount of arsenic excreted from day to day varies so much that a single twenty-four-hour sample cannot be considered indicative of the rate of excretion. Case 2 is very similar, showing a decrease of 36 per cent in the arsenic output. In Case 3 the urinary output was more than doubled when the thiosulphate was given, but the arsenic output was practically unchanged. In Case 4 the thiosulphate decreased the arsenic output for the four-day period about 25 per cent, while the urinary output was practically unchanged.

Cases 5 and 6 (Chart II) were cases of arsenical intoxication. It will be noticed in Case 5 that the arsenic output was not materially influenced, yet the patient improved. This is in keeping with Ravaut's report of beneficial results obtained by the use of sodium thiosulphate in dermatitis not due to metallic intoxication. Case 6 shows a definite reduction of the daily arsenic excretion.

DISCUSSION

Examination of the literature shows that sodium thiosulphate is considered by many to be an "ideal" antidote for arsenical poisoning and that most observers consider that its beneficial action is exerted by increasing the rate of arsenic elimination through the kidney. One is certainly convinced that clinical improvement has been seen in a great many cases[3] of intoxication, but so far no one has brought forth conclusive evidence to show how this beneficial result is obtained. Marples and Myers have attempted to explain the action of sodium thiosulphate on the assumption that a hypothetical "barrier" function of the cell has been interrupted. They say: "It may be reasonably assumed that the reason for the effectiveness of sodium thiosulphate in cases of arsenic intoxication is due to a stimulation of the processes which normally take care of arsenic but which have become sluggish on account of the toxic action of the metal." However, no statement is made as to what constitutes the "processes" which are stimulated, nor is any evidence offered to prove the presence of a "barrier" function in the cell.

The work of Voegtlein, Dyer, and Leonard[9] provides a much better explanation of the toxic action of arsenic. They have shown that the toxicity of arsenoxide is greatly reduced by glutathione and a number of other compounds containing an SH group. They say: "It is quite likely that arsenoxide reacts chemically with the reduced form of glutathione and perhaps also with some other SH compounds. * * * Reactions of this type would naturally reduce the concentration of the SH compounds of the cell in proportion to the amount of arsenoxide added. If the amount of arsenoxide furnished to the cell should exceed a certain limit, poisoning and death would necessarily follow as a result of the reduction of the absolute amount of SH compounds below the physiologic requirement." After quoting the work of Hopkins and Dixon to show the important part played by glutathione in the respiration of the cell they conclude that: "If glutathione really plays such an important part in the life of the cell, then it is not at all surprising that substances, such as arsenoxide, which react with the SH group of glutathione, should exert a toxic action. * * * In the final analysis arsenic would have to be considered as a poison which causes death of the cell by interfering with the oxidative processes governed by glutathione."

It is surprising that so few of the papers published have shown any attempt to follow the effect of thiosulphate on the rate of arsenic excretion. In the work reporting quantitative results one finds that a twenty-four-hour sample taken previous to the administration of the thiosulphate has been considered indicative of the rate of excretion. Kuhn and Reese[6] say: "In some cases the increase in the excretion was marked and in others it was slight. * * * the more marked increases were of slight duration and could not be maintained by subsequent doses of sodium thiosulphate." On examining the charts submitted by these investigators one is impressed with the fact that the administration of sodium thiosulphate is sometimes followed by a rise and sometimes by a fall in the arsenic elimination. Groehl and Myers[4] (page 693) submit a chart showing that the urine previous to the administration of the thiosulphate contained 8.18 mg. of arsenic. Two days after the thiosulphate injection had been started 31.48 mg. of arsenic were found. However, the arsenic output then fell to 4.01 mg. for two days even though the thiosulphate was being given. Further perusal of this chart shows a rise and fall of the arsenic content throughout the period of treatment which does not correspond with the administration of the thiosulphate. Most observers agree that less kidney damage is found after the administration of sodium thiosulphate. Is it not possible that the drug may act in two ways: (1) by forming an insoluble compound with the arsenic which is excreted more slowly and therefore does not reach the kidney in such high concentration and (2) by supplying available sulphur to the cells?

CONCLUSIONS

From the data submitted here one can conclude that in most instances the thiosulphate has markedly diminished the rate of arsenic excretion and has never increased it even when there is pronounced diuresis as in Case 3.

The experimental work on rabbits would indicate that the drug has some value in preventing kidney damage in chronic intoxication but that its value as an antidote when large amounts of arsenic have been ingested is questionable.

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STUDIES OF THE ACTION OF SODIUM THIOSULPHATE IN METALLIC INTOXICATIONS

II. The Effect of Sodium Thiosulphate Upon the Excretion of Lead*

By Arthur C. Curtis, M.D., and A. G. Young, Ph.D.

INTRODUCTION

SINCE the work of Ravaut,[1] numerous investigators[2] have used sodium thiosulphate in varying concentrations, both intravenously and by mouth, in the treatment of lead intoxications. Beneficial results have been claimed from its use. The mechanisms by which these effects occur have not been determined, though it has been inferred that sodium thiosulphate either converts an insoluble lead compound into a soluble, nontoxic, thiosulphuric acid derivative, of a complex lead proteinate[3]; or that it converts the soluble toxic metal into an insoluble, nontoxic compound.[4] If either premise is true, there should be some evidence of change in the amount of lead excreted in the urine and feces of a lead-poisoned animal after the administration of sodium thiosulphate. The investigation was devised to obtain an answer to this question.

It has been shown by Aub, Fairhall, Minot, and Reznikoff[5] that lead, whether administered by mouth, insufflation, or intramuscularly, is probably carried in the blood stream as a colloidal phosphate. Colloidal lead phosphate is then brought into contact with every body tissue; but marked retention occurs only in bones where it is deposited, under favorable conditions, as the tertiary phosphate. This latter compound is very sensitive both to changes

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in the H-ion concentration and to the blood calcium. Lead phosphate is released into the circulation when the blood calcium is low or when there is a state of acidosis or alkalosis. It remains stored when the blood calcium is high and the blood P_H normal. Lead is excreted by the gastrointestinal tract, kidney, bile, and skin, but the greatest excretion is in the feces.

It is, therefore, evident that diets high in absorbable calcium and low in acid precursors would tend to increase the storage of lead; whereas, diets which are low in absorbable calcium and which are acid-producing, would tend to increase the elimination of lead. It is then possible to compare the effect of sodium thiosulphate upon the excretion of lead when the diet is high in calcium and low in acid precursors or when the diet is low in calcium and high in acid-producing substances.

METHOD

Guinea pigs, rabbits, and white rats were used. They can be conveniently dealt with under three headings: A, B, and C.

The animals of Group A consisted of two guinea pigs. The animals of Group C consisted of five rats. The animals of Group A were fed a diet in which was incorporated small amounts of lead acetate. This diet was fed until each animal had ingested one hundred and fifty milligrams of lead acetate. The animals of Group C were fed a similar diet in which lead acetate was present in small amounts. The rats received this diet until each animal had eaten two hundred and two milligrams of lead acetate. All animals were then fed a high calcium lead-free diet for ten days to allow any lead present in the gastrointestinal tract to be excreted and to aid in storing the lead present in the circulation. This diet consisted of lettuce, milk, calcium lactate as a 1 per cent solution in milk, and bread. The animals of Group B consisted of two rabbits. Each was poisoned by a subcutaneous injection of 400 milligrams of lead acetate. All the animals of Groups A and B were put into separate metabolism cages, the bottom of each cage having been previously paraffined so that possible contamination from any lead present in solder would be avoided. All the animals of Group C were put into one metabolism cage and the urine and feces collected together under similar precautions used with the animals of Groups A and B.

Three-day specimens of urine and feces were collected and analyzed together for lead by the chromate method and by Fairhall's direct precipitation method.[6] During the period when the excreta were being collected for analysis of its lead content, two general types of diet were used. The first diet consisted of oatmeal, corn meal, and water. Table I shows such a diet to be low in calcium and high in acid precursors. The second diet consisted of milk and corn meal. Table I shows such a diet to be high in calcium and to have an excess in basic properties. A few leaves of alfalfa were given to all animals each day.

All animals were first fed diet No. 1 for a period of three days. The urine and feces were then collected and the cage was cleaned. Following this, they were given diet No. 1 plus 0.5 gram of sodium thiosulphate per kilogram of body weight, dissolved in their drinking water. At the end of

TABLE I

THE CALCIUM OXIDE AND PHOSPHORUS PENTOXIDE CONTENT OF MILK, CORN MEAL, AND OATMEAL EXPRESSED IN PER CENT PER 100 CALORIES*

FOOD	CaO	P_2O_5	EXCESS ACID	EXCESS BASE
KIND	PER CENT PER 100 CALORIES	PER CENT PER 100 CALORIES	GRAMS	GRAMS
Milk	0.456	0.599		5.0
Corn meal	0.004	0.080	1.5	
Oatmeal	0.030	0.216	3.0	

*Sherman.

TABLE II

THE EXCRETION OF LEAD DURING PERIODS OF THREE DAYS ON DIETS COMPARATIVELY LOW IN CALCIUM AND HIGH IN ACID PRECURSORS; HIGH IN CALCIUM AND LOW IN ACID PRECURSORS; AND WITH ADDITION OF SODIUM THIOSULPHATE TO EACH DIET

GROUPS	ANIMAL	DIET NO. 1		DIET NO. 2	
		LOW CALCIUM ACID FORMING	SAME DIET PLUS SOD. THIOSULPH.	HIGH CALCIUM LOW ACID	SAME DIET PLUS SOD. THIOSULPH.
	Number	Mg. of lead	Mg. of lead	Mg. of lead	Mg. of lead
A	Guinea pig No. 1	1.456	1.414	0.928	Lost
	Guinea pig No. 2	2.277	1.259	0.310	0.310* 0.690
B	Rabbit No. 5	1.380	1.367	1.311* 1.194	0.897
	Rabbit No. 8	1.242	0.745	trace	trace
C	Rats Nos. 1, 2, 3, 4, and 5	0.811* 1.187	0.828	0.345	0.552

*Determinations done on different three-day periods.

a three-day period the urine and feces were again collected and the cage was cleaned and reparaffined. All animals were then placed upon diet No. 2 for a three-day period and the urine and feces collected. They were then placed upon diet No. 2 plus the addition of 0.5 gram of sodium thiosulphate dissolved in milk. The urine and feces were collected at the end of three days on this diet. All three-day specimens were analyzed together for their content of lead.

DATA

It will be observed from Table II that the two animals of Group A excreted more lead on the first diet than they did on the second diet. The decrease produced by diet No. 2 amounted to 43 per cent in animal No. 1 and 86 per cent in animal No. 2. When sodium thiosulphate was added to diet No. 1 there was no appreciable change in the excretion lead in one animal and a decrease of 50 per cent was noted in the other animal.

Rabbit No. 5, of Group B, was pregnant while on Diet No. 2, and we feel that the results obtained during this period are not representative of the lead which might have been excreted had she not been pregnant, inasmuch as her calcium reserve would be very much depleted while the embryos were

developing and her blood P_H would tend to be more toward the acid side. These conditions more closely simulate those produced by the low calcium and high acid diet. Likewise, there is no appreciable change in her lead excretion while on diets Nos. 1 and 2. When sodium thiosulphate was added to the first diet, no effect was observed, but when added to the second diet the lead excretion fell approximately 25 per cent. Animal No. 8 showed a marked decrease in the excretion of lead; when shifted from the low to the high calcium diet the excretion fell about 40 per cent. The addition of sodium thiosulphate to the high calcium diet caused no change.

Group C shows approximately the same changes observed in Group A and Group B. There is a marked decrease in the lead excretion on diet No. 2 compared with the excretion of lead on diet No. 1. When sodium thiosulphate is added to the first diet, there is a slight decrease in the lead excretion, and when sodium thiosulphate is added to the second diet there is a slight increase in the amount of excreted lead.

DISCUSSION

It will be observed from the results compiled in Table II that diets which are low in calcium and comparatively high in acid precursors tend to favor the excretion of lead. Diets which are high in calcium and low in acid precursors tend to favor the retention of lead. Our work then confirms the work of Aub, Fairhall, Minot, and Reznikoff[7] in this respect. The diets used in this experiment were not extreme in either their calcium content or their acidic properties, and consequently the lead excretion was not great.

The addition of sodium thiosulphate to diet No. 1 seemed to lower somewhat the excretion of lead. We feel these results arise from the fact that sodium thiosulphate is an alkaline salt which, when added to a diet, would tend to decrease the acidity of the diet if the diet were potentially acid; or it would tend to increase the alkalinity of the diet if the diet were primarily alkaline. Diet No. 1 is an oat diet which has been shown by numerous investigators to be acid-producing.[8] Theoretically, the addition of sodium thiosulphate would tend to decrease the acidity of such a diet and would tend thereby to favor the retention of lead by the animal. Diet No. 2 is also an oat diet, but milk is present also, which is potentially alkaline. These two factors would tend to neutralize each other. Sodium thiosulphate added to the second diet would tend to increase the alkalinity and favor lead excretion. It will be noted that the excretion of lead is greater on a diet high in calcium to which has been added sodium thiosulphate than on a similar diet without sodium thiosulphate. The difference, however, is minor when compared to the amount of lead excreted on a low calcium, high acid diet.

The sodium thiosulphate was given in large doses by mouth to animals which had previously been fed lead, but which, at the time they were studied, had only absorbed lead present. They, also, were free of symptoms of lead poisoning when the drug was administered, so that the effect of sodium thiosulphate, either upon the symptoms of lead poisoning, or its effect when given to animals with ingested lead still present in the gastrointestinal tract is not determined.

SUMMARY

A review of the literature shows that no one has subjected the effect of sodium thiosulphate on lead intoxications to a carefully controlled study. Symptomatic improvement has been looked upon as evidence of the beneficial effect of the thiosulphate treatment. The recent work of Aub, Fairhall, Minot, and Reznikoff[7] has shown that substances tending to reduce an acidosis or alkalosis in cases of acute lead poisoning produce symptomatic improvement. They have also shown that diets high in calcium create a storage of lead in the bones and a rapid disappearance of all symptoms. One is impressed by the fact that no reference is made to the diet of patients being treated with sodium thiosulphate for lead intoxications when certain diets have been shown to play such an important part in the alleviation of symptoms. This omission alone is sufficient to cast considerable doubt on the value of the clinical evidence submitted to show that sodium thiosulphate is an antidote for lead poisoning.

In view of the work presented here and the lack of evidence to substantiate the claims made, we feel that there is reason to doubt the value of sodium thiosulphate as an efficacious antidote in lead poisoning until further study shows it to be advantageous. Our results would indicate that any other alkaline drug might give the same results as we obtained with sodium thiosulphate.

CONCLUSIONS

1. Our results confirm the work of Aub, Fairhall, Minot and Reznikoff, that diets which are high in calcium tend to decrease the excretion of lead, and diets which are low in calcium and acid-forming tend to increase the elimination of lead.

2. Sodium thiosulphate given by mouth in doses of 0.5 gram per kilogram of body weight to guinea pigs, rabbits, and rats previously poisoned by lead does not appreciably affect the excretion of lead.

3. The slight effect exerted by sodium thiosulphate upon the excretion of lead is due to its alkaline reaction and not to any ability to form soluble or insoluble lead compounds.

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RÔLE OF SPECIFIC AND NONSPECIFIC FACTORS IN ALLERGY AND ALLERGIC EQUILIBRIUM*

By Warren T. Vaughan, M.D., Richmond, Va.

SUCH terms as protein sensitization, anaphylaxis, immunity, and allergy have come into such general use that we are prone to assume an understanding of the fundamental processes in these conditions which is not justified by the facts. Many theories have been advanced to explain the phenomena of anaphylaxis and allergy, a great proportion of which have already passed into the discard. Even those theories which have come nearest to explaining all of the phenomena observed have met with the obstacle of new observations, unknown at the time at which the theory was proposed, observations which must perforce be fitted into the picture.

With the development of new methods of study old experiments are repeated, we might say with different instruments or measures, and new light is shed upon their mechanism. We formerly discussed immunology in terms of chemistry, later in the nomenclature of physical chemistry, and now from the point of view of the colloidal chemist. The biologic processes which we are attempting to study remain unchanged. It is our methods which vary, and unfortunately with the development of any radically new method of investigation, a new terminology is almost perforce introduced into the medical vocabulary, with the result that a single phenomenon masquerades often under many names.

The language of immunology is complex and vague chiefly because of our lack of knowledge of the processes involved. Without this knowledge we cannot use more exact terms. The immunologist "struggles with agents of unknown composition and measures results with a yard stick of uncertain accuracy. The physical chemist applies methods of greatest accuracy to materials of uncertain nature and to reactions which are modified by an almost infinite number of unknowable variables."

Our lack of clear understanding of the processes of anaphylaxis and allergy is a decided handicap. As Dean has well said, "Ignorance, however aptly veiled in an attractive terminology, remains ignorance."[1]

Theories are but the stepping stones of our progress toward knowledge. Without them we could not progress, nor must we remain too long upon one stone. Each theory has its temporal value, but it is deeply rooted in its own environment, and we must be searching for new ones farther along in the path of progress. Some stones are larger than others, and on the basis of a single theory we often cover rather a surprising distance. The end of the stone will, however, ultimately be reached, and we may be happy in the assurance that a new one will be found with a strong enough base for us to travel onward.

*Address before The Toledo Academy of Medicine, Toledo, Ohio, December 9, 1927.

It would appear that in the realm of immunology we have in recent years been traversing a surprisingly large stepping stone. Recent studies, especially in colloidal chemistry, have raised questions which the older theories of anaphylaxis and immunity appear unable to solve, and yet those theories have been the basis for all of our knowledge of clinical allergy as we see it in hay fever, asthma, urticaria, and several other conditions which I shall discuss later. Whether the theories have been true or false, they have been instrumental in aiding or relieving millions of suffering humanity. Whatever their future status, in their own times they were as near to the truth as could be approached.

In a paper on new viewpoints in allergy we must presume an understanding of the older viewpoints. For the sake of orientation, therefore, I shall review very briefly one theory of anaphylaxis, on the basis of which much of our present knowledge is builded, a theory which will almost necessarily require modification both in terminology and in context as the result of accumulating information, but one for which, so far as I know, no completely satisfactory substitute has been proposed. I refer to the explanation of anaphylaxis proposed by Victor C. Vaughan.[2]

It appears to be a biologic law that when a living cell comes in contact with a foreign protein, it will endeavor to elaborate a ferment or enzyme which will digest and destroy the protein. If no enzyme already exists for the particular protein, the cell will endeavor to manufacture one. The primary reason for this digestion of a foreign protein in the environment of a cell, is of course the acquisition of food material. Even in the human body this is equally true as far as the individual cell is concerned and the process would be as harmless as it is to the unicellular organism were it not for the fact that poisonous decomposition products are liberated, not in the outside environment but within the human economy itself.

When a foreign protein is introduced directly into the body, it will continue to exist as such until the body cells have elaborated an enzyme or antibody capable of digesting and destroying that protein. Whether it be a living microorganism or unorganized, as serum or egg albumen, it will persist in the blood, and, if living, may multiply, until sufficient enzyme has been produced to digest and destroy it. A good time, therefore, for positive blood cultures in typhoid fever is during the incubation period, before the symptoms of the disease have become manifest. Longcope has shown that horse serum injected into rabbits continues to exist as such until after the development of serum sickness.

Normally, the body tissues are not provided with specific ferments or enzymes for those proteins which should have been digested before absorption from the intestines. Once having acquired the ability to digest a protein, however, the cells retain that power, and can reproduce the specific enzyme whenever stimulated by the presence of that protein. From ten days to two weeks is usually required for the original elaboration of the antibody, but thereafter the introduction of the protein calls forth an almost instantaneous production.

Every protein appears to consist of a poisonous and of a nonpoisonous portion. The poisonous portion of the molecule is in all probability similar in all proteins. The nonpoisonous portion is different for every protein, and on this difference rests the specificity of the protein. The newly formed enzyme digests away the specific nontoxic portion of the molecule, thereby liberating the poisonous fraction. Symptoms of protein intoxication result from the liberation within the body of the poisonous portion. While the tissues are first learning to produce the antibody, protein is split slowly, so that at no one time are large amounts of poison present, and the symptoms are either absent or very mild. However, mild symptoms may follow even the first injection of foreign protein, as in the familiar cases of serum sickness. The slow liberation of poison prevents any serious reactions.

If, after the body cells have become sensitized—have learned to pour out rapidly the new destructive antibody—the antigen or foreign protein be again administered parenterally, the high resultant concentration of antibody produces such rapid destruction, and liberates such large amounts of poison in a short time, that serious symptoms result. In this way, typical anaphylactic shock may be produced. The symptoms are not necessarily respiratory. In the guinea pig, anaphylactic death is respiratory, and the symptoms are of asthma. In the dog, they are chiefly circulatory. All parts of the body are involved in anaphylactic shock, and the symptoms may vary accordingly.

In typhoid fever, when the tissues have at length become sensitized, the growth of the bacilli in the circulation has already produced such large amounts of protein to be digested that serious symptoms ensue. In malaria, the extrusion of the plasmodia from the red cells into the plasma, where digestion rapidly occurs, gives rise to the characteristic rigor and high fever.

In this paper I shall not enter into the controversy as to whether the mechanism of clinical allergy is identical with that of experimental anaphylaxis or whether it is an analogous but basically different reaction. It is difficult to explain some of the facts of clinical allergy in terms of existing theories of anaphylaxis, but nothing has been conclusively demonstrated either way, and it is possible that some future stepping stone will be found which will reconcile the points of apparent disagreement.

Meltzer, in 1910, first suggested that bronchial asthma may be an anaphylactic phenomenon. If this be true, we must presume that at some time the asthmatic patient first became sensitized by the penetration of the allergenic foreign protein into the body and that it was not until after this that the reintroduction of the same protein produced the symptoms of allergy. The objection has been raised, especially by Coca,[3] that we have been unable to prove the primary sensitization and that allergy is more often an hereditary manifestation. He also raises the objection that allergy has not been proved an antigen antibody reaction, that antibodies have not been found in the serum of allergics. There is no denying the hereditary factor in allergy, and it is true that some infants are born sensitized to certain proteins. The most recent work in this field has been contributed by Balyeat.[17] So far no analogy to this has been observed in experimental anaphylaxis. Passive

anaphylaxis may be transmitted from mother to offspring in guinea pigs, but the sensitization dies out in the progeny in a few weeks.

On the other hand, Coca's insistence upon the presence of an antigen-antibody reaction is, as Kolmer[3] points out, as yet unwarranted because: first, our methods of studying antibodies are still too crude, and second, antibodies may actually exist in the cells while remaining undemonstrable in the serum. Moreover, isolated instances have been recorded of allergic asthma developing after the original introduction of a foreign protein. Walker[4] has reported horse asthma persisting after the administration of horse serum and Sewall[4] has recorded a similar case in a laboratory worker after the injection of rabbit serum.

Besides this, there has been at least one case of passive transfer of sensitization in humans by the introduction of the serum of an allergic individual into a nonallergic. This may be interpreted as indicating the presence of antibodies in the blood. Ramirez[5] describes the case of a man who developed an attack of asthma for the first time in his life when he went driving in a horse and carriage shortly after having received a transfusion of 600 c.c. of blood for pernicious anemia. The patient then showed positive skin reaction to horse dander, and it was found that the donor of the blood was a horse asthmatic and was likewise sensitive to horse dander by skin test.

Indeed, Prausnitz and Küster[5] have developed a method of skin testing to be used, for example, in individuals with urticaria who give multiple nonspecific reactions to the skin test. This method consists in procuring serum from the allergic person, administering it intradermally to a nonallergic person, and, twenty-four hours later testing the latter with the various proteins, at the exact site of passive sensitization.

Of course, not all allergic patients are born such. I have had many patients developing symptoms even past middle life who have been sensitive to such common articles as wheat, egg, feathers, and the like, and who have had contact with them sometimes for half a century or more before developing any symptoms suggestive of allergy. I have seen sensitization to one protein develop in individuals previously nonsensitive to that protein, although they were sensitive to others.

Why do some people develop hay fever and asthma while others with similar local pathology and similar protein exposures do not?

With inhalent proteins it is possible that mechanical abnormalities in the nose play a part. It has been shown that proteins, such as pollen protein may be digested to a certain extent by the normal nasal secretion. With defective aeration, as behind a nasal spur, abnormal quantities of mucus collect, and here it is conceivable that protein is absorbed through the nasal mucosa. There is also evidence that the absorption of undigested protein through an apparently intact mucous membrane renders that surface more permeable for subsequent absorption of protein. Sewall has produced sensitization in guinea pigs by the application of horse serum to the nasal mucous membrane. Therefore, an overdose or massive dose of protein applied to the nasal mucosa might overcome the resistance of any individual irrespective of local pathology.

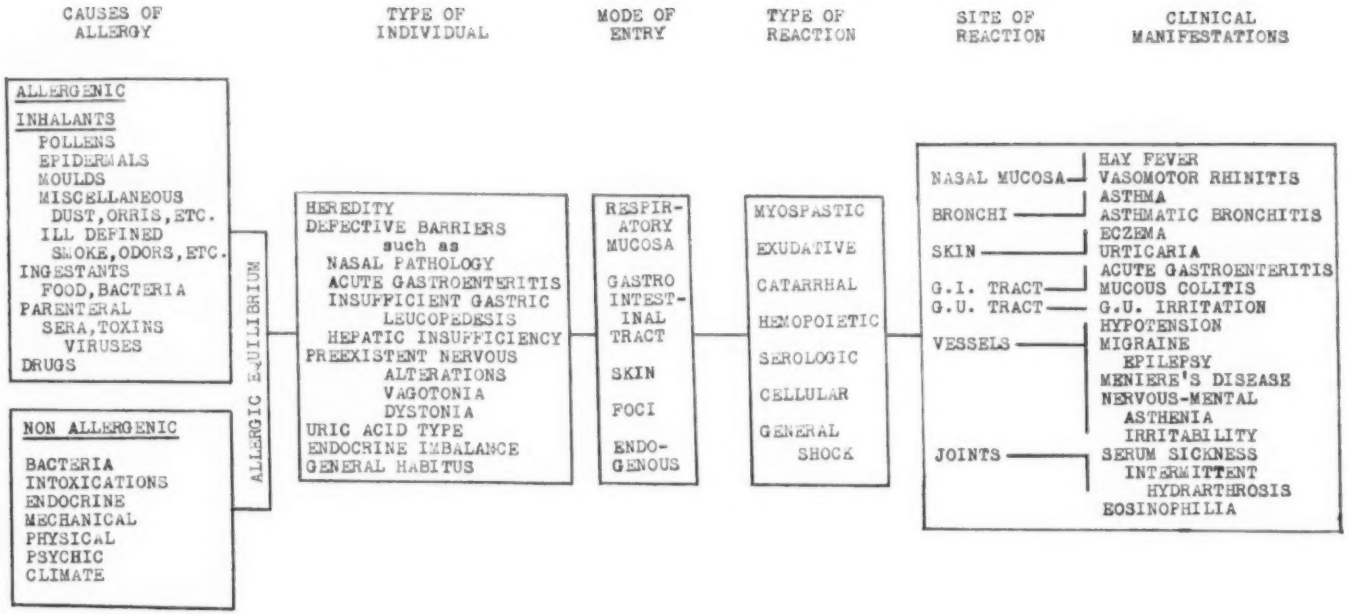
Most of the work on experimental anaphylaxis has been done on guinea pigs. It is true that there are many points of difference between human allergy and guinea pig anaphylaxis; but it is equally true that there is as great a difference between anaphylaxis as observed in guinea pigs and that observed in other experimental animals, such as the rabbit, dog, and horse. The chief recognizable phenomenon in experimental anaphylaxis is smooth muscle spasm. In guinea pigs the bronchial musculature is most active, although as is well known, the uterine muscle also contracts. In anaphylactic rabbits there is little spasm of the bronchial muscles but pronounced spasticity of the muscles of the pulmonary arteries and arterioles. Consequently the symptoms are quite different. In dogs the smooth muscle preponderance appears to be in the portal circulation, and the symptoms of anaphylaxis are gastrointestinal rather than respiratory.

The outstanding feature in dogs is a pronounced fall in blood pressure. With this, but apparently not dependent on it, there is an increased flow of lymph and a severe local reaction in the intestines. This amounts practically to an acute enteritis with edema and petechial hemorrhages in the mucous membrane of the gut, the lumen of which is often filled with mucus mixed with blood. Weil[6] suggests that the differing symptoms in these three types of laboratory animals are due primarily to differences in quantitative distribution of smooth muscle. He finds an astonishing development of the bronchial musculature in normal guinea pigs, while the pulmonary arteries of rabbits present a remarkable degree of muscular development. The walls of the hepatic veins of dogs differ from those of the other animals in showing again a high muscular development. This suggests attractive speculation as to why some allergic persons develop hay fever or asthma, others headaches or the symptoms of an acute gastroenteritis, while others show predominantly dermal manifestations.

I have found protein sensitization to be a factor in such apparently diversified and unrelated conditions as hay fever, vasomotor rhinitis, asthma, eczema, urticaria, acute gastroenteritis, mucous colitis, hypotension, migraine, certain mental states, serum sickness and intermittent hydrarthrosis. Other immunologists have added still more conditions to this list. It is certainly conceivable that with different types of reacting individuals, with differing modes of entry of the foreign protein into the body, with different types of reactions as enumerated on the accompanying chart and with differently located preponderances of the reacting tissues within the body, the allergic explosion will manifest itself in a wide variety of clinical forms.

I have prepared the accompanying chart to correlate the many etiologic factors and clinical manifestations of allergy. Study of the chart will show very easily how many factors may be at work and how many symptoms may result even though the basic underlying conditions remain the same. The chart also illustrates diagrammatically my theory of allergic balance or equilibrium.

The immediate exciting causes of allergic reactions, whatever their type, are most varied. The direct etiologic agents are usually specifically allergenic,


CAUSES OF ALLERGY
TYPE OF INDIVIDUAL
MODE OF ENTRY
TYPE OF REACTION
SITE OF REACTION
CLINICAL MANIFESTATIONS
ALLERGENIC
INHALANTS
POLLENS
EPIDERMALS
MOULDS
MISCELLANEOUS
DUST, ORRIS, ETC.
ILL DEFINED
SMOKE, ODORS, ETC.
INGESTANTS
FOOD, BACTERIA
PARENTERAL
SERA, TOXINS
VIRUSES
DRUGS
NON ALLERGENIC
BACTERIA
INTOXICATIONS
ENDOCRINE
MECHANICAL
PHYSICAL
PSYCHIC
CLIMATE
ALLERGIC EQUILIBRIUM
HEREDITY
DEFECTIVE BARRIERS
such as
NASAL PATHOLOGY
ACUTE GASTROENTERITIS
INSUFFICIENT GASTRIC
LEUCOPEDESIS
HEPATIC INSUFFICIENCY
PREEXISTENT NERVOUS
ALTERATIONS
VAGOTONIA
DYSTONIA
URIC ACID TYPE
ENDOCRINE IMBALANCE
GENERAL HABITUS
RESPIRATORY MUCOSA
GASTRO INTESTINAL TRACT
SKIN
FOCI
ENDOGENOUS
MYOSPASTIC
EXUDATIVE
CATARRHAL
HEMOPOIETIC
SEROLOGIC
CELLULAR
GENERAL SHOCK
NASAL MUCOSA
HAY FEVER
VASOMOTOR RHINITIS
BRONCHI
ASTHMA
ASTHMATIC BRONCHITIS
SKIN
ECZEMA
URTICARIA
G.I. TRACT
ACUTE GASTROENTERITIS
G.U. TRACT
MUCOUS COLITIS
G.U. IRRITATION
VESSELS
HYPOTENSION
MIGRAINE
EPILEPSY
MENIERE'S DISEASE
NERVOUS-MENTAL
ASTHENIA
IRRITABILITY
JOINTS
SERUM SICKNESS
INTERMITTENT
HYDRARTHROSIS
EOSINOPHILIA

that is, foreign protein. However, nonspecific, nonallergenic factors may also be at work. Usually both types play a part, although the former plays a preponderant one.

The more we learn from our study of these various clinical conditions as allergic phenomena, the greater has been the number of cases that we have been able to relieve or completely cure by specific therapy; but at the same time the more closely have we been forced to a conclusion that allergy and allergic disease cannot be explained in its entirety purely on a basis of protein sensitization. We find asthmatic or eczematous individuals clearly sensitive by skin test to several different proteins, some of which produce no symptoms after exposure. If an individual is sensitive to two proteins apparently in equal degree, why does but one cause symptoms? How are we to account for Rackemann's observation that 10 per cent of normal healthy children who have had no evidence of allergy are sensitive to one or more foreign proteins? It is true that 13 per cent of these did develop allergic manifestations after varying intervals. But why had they not developed them at the same time that they developed the capability of giving positive skin reactions, especially when they were coming in sometimes daily contact with the offending protein?

Take a group of individuals all sensitive, among other things, to the protein of wheat, all relieved by the elimination of wheat from the diet and all or practically all having experienced recurrence of symptoms when again eating wheat, thereby proving the etiologic position of this protein. One has bronchial asthma, another recurrent urticaria, several have eczema, one involving the back of the neck, another the left hand and fingers, another the perianal region, and still another with a generalized eczema involving practically the entire body. Why the *difference in the localization* within the body when all are sensitive to the *same protein* and apparently in *like degree* and when all are coming into regular *daily contact* therewith?

In the case of eczema we may explain this as due to the added action of a *nonspecific* factor. Thus, mechanical irritation, the rubbing of the collar on the neck, moisture and friction in the perianal region, or mechanical trauma of the hand. Likewise, we may think of bronchial asthma as occurring because of some inherent diminished resistance of the mucosa of the respiratory tract, from preexisting nonspecific respiratory infection, etc., but that this is not the entire explanation is indicated by observation of the same varieties of localization in otherwise healthy infants, none of whom have been subject to precedent disease.

Again, with an individual sensitive to some protein with which he comes in *daily* contact, why is it that at times he remains symptom-free, the disease manifesting itself only in attacks?

Two answers present themselves to explain in part the various questions raised. First, in all probability *nonspecific factors do act*, often, coincidently with specific agents, both in the precipitation of allergic symptoms and at the same time in determining the localization thereof. Second, even this conception of an interaction between specific and nonspecific causative factors will not answer all of the questions raised, because *our conception of the patho-*

genesis of allergy is still incomplete. We now discuss it purely in terms of protein sensitization. The true conception will probably not be arrived at until we have progressed to where we can discuss it in terms of intracellular activity, in terms of the chemistry or physical chemistry of the individual cells located in various regions of the body. Thus, localized urticaria from heat or light, or asthma from exposure to cold or wind, must ultimately be explained in terms of cellular activity.

On the other hand, take the sensitive child who has never had symptoms from exposure to a protein to which he is sensitive. Take the individual sensitive to a protein with which he comes in daily, almost constant, contact but who manifests symptoms therefrom only at intervals. I look upon these individuals during the intervals of freedom as being in what I have termed a *balanced allergic state.* The patient is sensitive but by some mechanism still obscure, maintains a normal balance. It is useless in the present state of our knowledge to attempt to localize the mechanism by which this is accomplished. We may speak of the detoxicating action of the liver or of the activities of the endocrine system, but so far the part which they play is purely hypothetical. At any rate, the individual receives the allergenic protein within the system and disposes of it somehow without developing any of the various allergic manifestations; but allow some additional factor to overthrow this governing mechanism and symptoms will ensue. The additional factor may be *either specific* or *nonspecific.* It may be an overdose of the same allergen. Thus, a child in my experience, sensitive to the protein of chocolate, can ingest small amounts with impunity while large amounts overthrow the balance and precipitate asthma. A patient with eczema of the hands, sensitive to wheat protein, noted that her symptoms cleared up on a postwar visit to Germany, where the amount of available wheat flour was distinctly limited.

An individual may be sensitive to several proteins, as for example, foods, and satisfactorily metabolize one of them but develop symptoms when the mechanism becomes overwhelmed by the simultaneous ingestion of two or more.

A woman with autumnal hay fever due to ragweed and without symptoms of other allergic disease, aside from recurrent urticaria, experienced but 25 per cent improvement in her hay fever following pollen desensitization. She was then found sensitive to several foods, including wheat and egg, and upon eliminating these from the diet, not only was her urticaria relieved, but, which is intensely interesting, her hay fever disappeared completely.

These are examples of allergic unbalance, following the action of specific etiologic agents.

Again, the protective mechanism may become deranged through *nonspecific* causes. In eczema particularly we observe outbreaks associated with unusual constipation. The effect of teething on asthma or eczema is well known. Intercurrent infections and focal infections may act apparently nonspecifically. It is still a moot question as to whether bacterial protein may act at the same time as a *specific* allergen.

Intestinal upsets of various sorts, prolonged exhaustion, emotional upheaval, may become nonspecific provocative factors. A boy of thirteen with

perennial asthma, relieved for a period sufficiently long to be conclusive, by the removal of the offending food proteins and removal from association with feathers, had an altercation with his friends. One boy starts throwing stones at him. The patient has to protect himself behind a telephone post. Within ten minutes he is in a violent attack of true bronchial asthma. He has eaten nothing and his environment has not changed. A child five years old, sensitive to the proteins of wheat, strawberries, and chocolate, who has been symptom-free for two years on dietary treatment, falls by accident into a swimming pool. He is rescued at once, scarcely having had time to sink beneath the surface of the water. An attack of asthma follows shortly. This may have been due to fright or to sudden cold, but whatever the cause, it was nonspecific in nature.

Many an individual, having discovered those articles to which he is sensitive, can remain symptom-free until he develops some intercurrent infection, such as acute coryza, which appears to overthrow the allergic balance.

The effect of mechanical or chemical irritation is experimentally shown in the work of Auer.[7] Xylol applied to the ears of *nonsensitized* rabbits produced no great degree of inflammation. If, on the other hand, xylol was applied to *sensitized* animals which were then given otherwise ineffective doses of antigen, local necrosis occurred. He concluded that the inflammatory action of xylol caused accumulation of effective amounts of antigen in the locality affected. The practical application of this will be seen, especially in eczema.

Nonallergic pathologic conditions in the body may play a part. Nasal polyps have been recognized as etiologic agents for years. Hernia has been mentioned. Abdominal allergy is more likely to occur in individuals in whom there is some other pathology in the intestinal tract. Disease of the endocrine system plays a part, the extent of which we do not yet know. Thus, the case has been reported of a woman who developed bronchial asthma shortly after the production of an artificial menopause. She was treated over a period of practically two years with the elimination of those proteins to which she had been found sensitive, without benefit whatsoever. Three days after the exhibition of ovarian and mammary extract, it is reported that her asthma ceased. I have the record of a middle-aged woman, sensitive to several food and epidermal proteins, who did not respond to removal of the allergens, to autogenous vaccine, to peptone treatment, or to the administration of calcium chloride intravenously, but who promptly recovered, following the administration of thyroid and parathyroid extracts, the former in relatively large doses. It is of interest to note that her attacks came usually with the catamenia.

Many causes may interact in the production of symptoms, but the basis of treatment is most assuredly the sensitization test. The nose and throat man can often return a patient to allergic balance by removal of foci, polyps, and the like. The dermatologist can relieve eczema by the removal of local irritation and by the application of soothing ointments. The pediatrist can do likewise by general dietary readjustments, but they have many failures, and the patient, still allergic, is but in a temporary state of equilibrium which may again be overthrown by any of several factors. I may say without great

fear of contradiction that more consistently good and permanent results have been obtained by the application of the sensitization tests and resulting protein avoidance than by any other method. Furthermore, if desensitization or protein avoidance for a sufficiently long period actually enables the tissues to recover from their sensitization, this method of treatment is actually curative. I have observed positive skin tests become negative after avoidance.

What type of individual will manifest allergy? Many persons are exposed to all of the factors which we have mentioned, both allergenic and nonallergenic, and yet never develop the disease. Heredity undoubtedly plays a chief rôle. Predisposition to allergy appears to follow the Mendelian laws of heredity. It need not be always hay fever or asthma or eczema or migraine in each generation but in the majority of allergeric patients there is a clear cut and usually multiple family history, some having had asthma, others hay fever, or again urticaria, eczema, or migraine.

Defective barriers against the entrance of foreign protein probably play a part, particularly where there is an hereditary predisposition. I have already mentioned how local nasal lesions may play a part, and to this I must add pathology in the throat or lungs, such as bronchitis, gas poisoning, tracheobronchial lymphadenitis, and the like.

Acute gastrointestinal upsets have been clearly demonstrated to favor the absorption of undigested protein. Schloss and Worthen[8] have investigated the permeability of the gastroenteric tract in infants. It was shown by precipitin and anaphylactic tests applied to the urine that the intestinal tract of normal infants is impermeable to undigested foreign protein but that in gastrointestinal disturbances proteins may be absorbed undigested or partially digested and appear in this state in the urine. Hettwer and Kriz[9] have sensitized guinea pigs by the direct injection of horse serum into a clamped loop of small intestine. They have produced the symptoms of anaphylaxis in sensitized pigs by the direct injection of serum into a ligated loop. Indeed, large amounts in the unligated intestines produced anaphylactic symptoms. These authors concluded that increased intraintestinal pressure was a factor in promoting the absorption of protein and suggested that this pressure might be increased in certain clinical conditions, such as stasis.

Among the factors which may interfere with normal digestion and absorption may be mentioned insufficiency of the digestive secretions, such as gastric achylia. Loeper[5] finds that the introduction of any soluble substance into the stomach provokes a migration of leucocytes, chiefly polynuclears, into the cavity of the stomach. The extent of this reaction may be determined by the ingestion of 1 per cent peptone solution followed by leucocyte counts of the gastric contents at stated intervals. Loeper believes that one predisposing cause for the assimilation of undigested protein is a deficient gastric leucopedesis.

The old family physician spoke glibly of torpid liver. The clinical investigator mocked the phrase and other similar expressions; but empiricism often paves the way for rational medicine, and the liver appears today to be coming back into its own. Diagnostic liver function methods are still too imperfect to be of great value, and there is still far too much that we do not

know of the functions of this organ; but we do have ample evidence that the liver does play a part in certain immunologic processes and in nitrogen metabolism.

Manwaring and Crowe[10] have found that the liver in dogs is an important organ in the production of anaphylaxis. With the liver out of the circulation, anaphylactic shock does not occur in dogs. Opie[11] remarks that preliminary perfusion of the liver destroys the power of this organ to produce anaphylaxis. Egg white injected into the circulation appears in the urine sooner when introduced through an ear vein than through a mesenteric vessel. In the rabbit, therefore, the liver appears to hold back egg white. Furthermore, it does appear in the bile. In guinea pigs which have been previously sensitized against egg white, this protein causes quicker death and with smaller doses when injected directly into the systemic circulation than when introduced into the portal system. Bacteria introduced into the circulation are removed by the Kupffer cells and by the polyhedral cells of the liver. The coincident introduction of the serum of an animal immunized against these bacteria increases the rapidity of their removal by the liver. Jones[12] finds a greater concentration of agglutinin to hog cholera in the liver than in other organs. Maurer and Gatewood[13] record that the livers of sensitized rabbits remove phenoltetrachlorphthalein from the circulation more rapidly than do the livers of nonsensitized rabbits.

The liver removes many drugs from the circulation, such as phosphorus, chloroform, the halogen compounds, hyoscyamine, and atropine. Atropine introduced into the portal circulation appears in the systemic circulation in only one-twentieth the concentration. Opie records that the liver cells remove particulate matter, such as manganese dioxide, broken down red cells and even colloidal suspensions.

The part which the liver plays in nitrogen and protein metabolism is well known. Mann found that in dogs in which the liver was entirely removed, urea was no longer produced and uric acid was no longer destroyed. Ammonia which is normally removed from the portal circulation in the liver, passed through into the systemic circulation. Major has shown that liver extract appears to have a definite effect upon the metabolism and excretion of the guanidine bases. Toxic substances, such as benzoic acid and camphor, are synthesized into nontoxic substances in the liver. Thus, we have abundant evidence of regulatory and detoxicating processes in the liver. Many functional tests have been proposed, but none has been found convincingly satisfactory.

Widal, Abrami and Iancovesco have suggested the hemoclastic or colloidoclastic reaction as a direct test of the ability of the liver to remove foreign protein from the portal circulation. Peptone shock is accompanied by a leucopenia, fall in blood pressure, and prolonged coagulation time. These authors and others find this type of reaction present after the enteral administration of peptone or milk, in certain individuals with liver disease and in some allergic patients. This test has therefore been proposed as a measure of liver function, especially in allergy. Unfortunately the results are not constant in any one type of disease, and the reaction requires further elucidation.

Galup and Ségard[5] bring out that the phenomenon might equally be explained as a reflex vasomotor manifestation, resulting from contact of milk with the gastric mucosa in vagotonic individuals.

Is there an allergic diathesis? Is there a nervous predisposition to such diseases as asthma or migraine? Is this a basic factor or is it coincidental? Allergy appears to occur especially in the vagotonic type of individual. Vagotonia is, however, by no means a constant finding in allergic patients. Perhaps more frequently we observe a dystonia, an imbalance between the sympathetic and parasympathetic innervation with alternating dominance of one or the other nerve groups.

Some writers, particularly those in France, believe there is some association between a uric acid diathesis and the allergic state.[5] They mention a frequent occurrence of gout with asthma and state that the onset of an asthmatic outbreak may sometimes be foretold by the disappearance of urates from the urine.

There is much clinical evidence of an endocrine factor in the allergic disturbances, such as onset or termination at or near adolescence or the menopause, the relationship of attacks to the catamenia, and the result of opotherapy in selected cases.

I have said that smooth muscle spasm is the outstanding recognizable experimental evidence of anaphylactic reaction. This myospastic reaction is, however, accompanied by other reactions more difficult to follow. All of these reactions probably play a part in most allergic explosions. Huber and Koessler,[14] for example, have shown that the pathology of bronchial asthma consists not only of a smooth muscle hypertrophy in the bronchial tree but in a definite exudation of serum with a catarrh of the secreting epithelium. The hemopoietic reactions are eosinophilia and sometimes leucopenia. Coca[15] has emphasized the importance of the state of activity of the reactive tissue, or "shock organ," at the time of contact with the allergen or atopen, no matter whether this tissue is located in the bronchi, the nasal mucosa, the skin, or elsewhere.

Whether or not we grant the presence of antibodies in allergy, the blood serum plays a great part in the reaction. Call it antigen-antibody reaction, disturbance of colloidal equilibrium, or whatever the prevailing fashion of the day, the fluids of the body play some part even though they may not be the exact site of the allergic reaction. I will venture to prophesy, however, that when the last word has been said on anaphylaxis and allergy, these phenomena will be discussed in terms of the vital activity of the individual living cells.

Having seen how many different types of factors may interact to produce allergic symptoms, how the functional state of the reacting body plays a part, how the direct causative agents may enter at various places in the body, and how the reaction is expressed by a variety of types of responses, one should have little difficulty in understanding how the clinical reactions may take on a wide variety of forms. On my chart I have enumerated those disease conditions which, in my own experience and in the experience of others, appear often to be influenced by the allergic state. I do not mean to say that allergy

is the cause for all of these diseases. Indeed, my entire thesis has been that it is but one of many causes, sometimes the most important, sometimes perhaps the least important, but I will say that treatment instituted on the basis of positive sensitization reactions and perhaps modified in accordance with other factors which we have discussed has often enabled me to give relief to sufferers from any of this group of diseases, where other methods have previously failed.

Not all of my cases of migraine, for instance, are allergic. I have found an allergic factor in 37 per cent of migraine cases.[15] The remainder may or may not be allergic, but with this 37 per cent I obtained relief where other treatment had failed. Fifty-five per cent of my eczema series[16] were relieved by protein avoidance alone. The other 45 per cent may not have been allergic, but it is worthy of note that in the series that did not experience relief, there were nearly as many positive skin tests as in the other series. This was also true for the personal history of allergic diseases other than the eczema and for family histories of allergic disease. It seems probable, therefore, that allergy was also a factor in a number of these poor result cases, but that some of the other factors which we have discussed prevented satisfactory results.

I make no pretense that the chart which I have presented is complete or inclusive. Exception might well be taken to some of my classifications, but I do feel that it shows more clearly than any prolonged discussion how clinical allergy is a disease of many ramifications, how many apparently unrelated or even contradictory observations may be correlated into the clinical picture, and how the clinical manifestations may take on any of a wide variety of forms.

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REPORT OF A CASE OF RENAL DIABETES ASSOCIATED WITH DIABETES MELLITUS*

By J. A. Curran, M.D., and C. A. Mills, M.D., Peking, China

THE term "renal diabetes" properly refers only to those instances of glycosuria accompanied by a normal or subnormal blood-sugar level. When a discussion of this condition arises, one is already cautioned to be certain that the condition be not really diabetes mellitus, and if any disturbance of glucose utilization be present, the conclusion seems to be that a diagnosis of renal diabetes is not justified. We feel that such an attitude is not correct, and we present this case as a justification of our stand. We believe that an excretion of sugar by the kidneys of a patient with diabetes mellitus, at a time when that patient's blood-sugar level is normal or subnormal, just as truly indicates the presence of renal diabetes as would a similar excretion in a patient without diabetes mellitus.

In cases of diabetes mellitus of long standing it is usually observed that the kidney threshold for glucose rises and that diabetic patients may often have a fasting blood sugar of 200 mg. per 100 c.c. or over, and still show no glycosuria. We have had one patient this year whose fasting blood sugar rose to 250 mg. without the presence of glycosuria. We make these statements to emphasize further the importance of diagnosing renal diabetes in the presence of diabetes mellitus when sugar excretion occurs at a normal or low blood-sugar level. The bearing of such a diagnosis on the diabetic treatment is evident, since the urinary sugar is usually accepted as the most convenient guide. We shall discuss these points after presenting the details of the case.

L. T. Y. (P. U. M. C. Hospital No. 14607), a Chinese man, aged fifty-six, had had polydipsia and polyuria for twenty-five years, although otherwise in good health. A urinary examination in June, 1926, disclosed the presence of sugar, but no other symptom of diabetes was present. He was admitted to the P. U. M. C. hospital on August 24, 1926. Physical examination was negative. The blood pressure was normal. Renal function test showed a phthalein excretion of 67 per cent in two hours. Blood and urine examinations were negative, except for glucose abnormalities. At this time his fasting blood sugar was 200 mg. (Folin and Wu method) and the urinary output (without dietary control) was in the neighborhood of 80 gm. in twenty-four hours. After nine days on a 2000 calorie diet with a fatty acid-glucose ratio of 1.5, and thirteen days on 1880 calories with a ratio of 1.6, the glucose excretion gradually fell to between 6 and 8 grams per day, accompanied by a loss of body weight from 90 to 85.6 kilos. At the end of this period his fasting blood sugar was 71 mg., although at all periods of the day glycosuria was present. Glucose tolerance test, performed at this time, gave the following results:

	Fasting	*After ½ hour*	*1½ hours*	*3 hours*
Blood sugar in mg.	71	166	181	133
Urinary sugar per cent	0.9	2.7	8.3	5.5

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Administration of from 6 to 7 units of insulin daily made no difference in the sugar excretion. He left the hospital against advice, and we later learned that the physician in charge of his treatment could get no satisfactory effect of insulin, using even up to 40 units daily. He was readmitted for study November 29, 1926, with a blood sugar of 133 mg. and a sugar output in the urine of 27 grams in twenty-four hours. In the course of five days on a 2000 calorie diet (ratio 2.0), the sugar output fell to 7 grams per day, and on 2140 calories (ratio of 2.47) the output was maintained between 4.4 and 7.3 grams for two weeks, while the body weight fell from 87.4 to 86.1 kilos. The administration of 15 units of insulin daily for five days gave a very slight effect, keeping the output between 3.9 and 4.9 grams per day. Fasting blood sugar after one week of this diet, and before giving the 15 units of insulin daily was 66.6 mg.

A sugar tolerance test performed at the end of this dietary period, using 0.2 c.c. of blood from finger puncture for each determination gave:

	Fasting	*After ½ hour*	*1½ hours*	*3 hours*
Blood sugar in mg.	135	309	250	153
Urinary sugar per cent	0.43	3.8	5.0	4.1

The patient was discharged from the hospital, with advice to his physician to allow him an adequate diet and to use insulin sufficient to keep the urinary output approximately 8 grams a day. With the diagnosis of renal diabetes associated with the diabetes mellitus, and the knowledge of the amount of urinary sugar to be accounted for on that basis, the treatment now became satisfactory to both physician and patient.

DISCUSSION OF THE CASE

We had here a patient with definite findings of diabetes mellitus, as evidenced by the blood-sugar curve in the glucose tolerance test and by the marked lessening of urinary sugar on reduction of glucose intake or with insulin administration. However, on a low food intake, with or without insulin, the sugar excretion continued at times when the blood-sugar level was normal or below normal (71 mg., 66.6 mg., 111 mg.). On the basis of these findings we made a definite diagnosis of renal diabetes associated with diabetes mellitus, and advised that, in the treatment of the latter disease, the urinary sugar accountable for by the former should be disregarded. The lack of any evidence of nephritic changes other than the reduced glucose threshold places this case with the less frequently found type of renal diabetes which is not associated with chronic nephritis.

A SPECIES OF ESCHERICHIA FROM GASEOUS INFECTION*

By Frederick W. Shaw, M.D., Richmond, Va.

WEINBERG and Seguin,[1] from a study of 126 cases of gaseous wound infection other than tetanus, found six cases of the phlegmonous type which yielded aerobes only. It is thus concluded that aerobes are apparently able to produce gaseous phlegmons, but the number of such cases is small. The organism to be described in this paper was isolated in pure culture from the deep structures of the lesion. It produced a gaseous infection in rabbits, from the lesions of which it was recovered in pure culture.

CASE REPORT

R. G., white male, entered the Hospital Division, Medical College of Virginia. The left patella was fractured, and there were numerous abrasions over the lower and upper extremities. There was a laceration of the inner aspect of the left lower thigh. Four days later the patient was delirious; his temperature was 103° F. and his pulse 130. Examination of the laceration at the inner side of the left knee showed an inflamed area of about 6 cm. Crepitation was present and extended to the groin. The left leg was amputated at the hip.†

A gross description of the leg showed a wound 6 cm. in length penetrating the skin on the lateral aspect of the knee. The subcutaneous tissue of the anterior and lateral aspect of the leg were crepitant. On section the subcutaneous tissue and the intermuscular fascia of the region were filled with an extensive hemorrhagic edema. There was definite involvement of the superficial muscles and at approximately the same line some of the deep muscles were more involved and appeared dark and soft. There was no odor.

A variety of culture media were inoculated from the deep structures. Gas was given off from the anaerobic meat media in six hours. All of the culture media showed a Gram negative, nonspore-forming rod. No other organism was observed. Subcutaneous inoculations into guinea pigs killed them in twenty-four hours. Autopsy showed a septicemia. Subcutaneous inoculations into rabbits showed a definite area of beginning crepitation at the area of inoculation, beginning in about forty-eight hours. On the sixth day the lesions showed a crepitant area of about 5 cm. Incision of this area revealed an inflammation of the underlying muscles accompanied by the presence of gas. There was no odor. The original microorganism was isolated in pure culture from the heart's blood of the guinea pigs and from the lesions in the rabbits.

BACTERIOLOGY

A description of the organism follows:

Short rods; motile; Gram-negative; no spores.
Aerobic and facultative anaerobic.
Gelatin not liquefied.
Agar colonies: whitish-gray, slightly spreading.

*From the Department of Bacteriology, Medical College of Virginia.
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†The patient's temperature reached normal in six weeks, and he was discharged two weeks later.

TABLE I

CULTURAL REACTIONS OF ESCHERICHIA EMPHYSEMATA AND RELATED ORGANISMS

	MOTILITY	GELATIN	SERUM	MILK	LACTOSE	SACCHAROSE	DULCITOL	MANNITOL	DEXTROSE	MALTOSE	DEXTRIN	RAFFINOSE	ARABINOSE	ADONITOL	RHAMNOSE	XYLOSE	INULIN	SORBITOL	GALACTOSE	LEVULOSE	INOSITOL	SALICIN	AMYGDALIN	GLYCEROL	TREHALOSE	INDOL	V.-P.
E. acidi-lactici	O	O	O	AC	AG	O	O	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	AG	O	O				P	O
E. coli	+	O	O	AC	AG	O	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	O	AG	AG	AG	O	AG	O		AG	P	O
E. communior	+	O	O	AC	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	O	AG	AG	AG	–	–	–		AG	P	O
E. coseoroba	O	O	O	AC	AG	AG	O	AG	AG	AG	AG	AG	AG	O	–	AG	–	AG	AG	–	–	–	–		–	P	O
E. emphysemata	+	O	O	AC	AG	O	O	AG	AG	AG	O	O	AG	O	–	AG	O	O	AG	AG	O	O	O		AG	P	O
E. enterica	O	O	O	O	AG	O	AG	AG	AG	AG	AG	–	AG	O	–	–	O	AG	AG	AG	–	–	–		–	P	O
E. grünthali	+	O	O	AC	AG	O	O	AG	AG	–	AG	AG	AG	O	–	–	O	AG	AG	AG	O	–	–		–	P	O
E. neapolitana	O	O	O	AC	AG	AG	AG	AG	AG	AG	AG	AG	AG	O	AG	AG	O	AG	AG	AG	O	AG	O		AG	P	O
E. paragrünthali	+	O	O	AC	AG	O	O	AG	AG	AG	AG	AG	AG	O	AG	–	O	AG	AG	AG	O	AG	O		AG	P	O
E. pseudocoloides	+	O	O	AC	AG	AG	O	AG	AG	AG	AG	AG	AG	O	AG	–	O	–	AG	AG	O	AG	O		AG	P	O
E. vekanda	+	O	O	A	AG	O	AG	AG	AG	AG	O	O	AG	AG	AG	–	O	AG	AG	AG	O	O	O		AG	P	O

Key: O, no reaction; A, acid; C, coagulated; G, gas; p, Indol produced; tvariable.

Broth: turbid with sediment. No pellicle.

Serum not liquefied.

Milk: acid and coagulated.

Indol is formed.

Nitrates reduced.

Lead acetate not blackened.

Acetyl-methyl-carbinol not formed.

Acid and gas in lactose, mannitol, dextrose, maltose, arabinose, galactose, levulose, rhamnose, glycerol, xylose, and trehalose.

No acid or gas in saccharose, dulcitol, dextrin, raffinose, adonitol, inulin, sorbitol, inositol, salicin or amygdalin.

This microorganism is readily differentiated from Escherichia coli by its inability to ferment dulcitol. It closely resembles Escherichia grünthali (Morgan) Castellani and Chalmers, and Escherichia paragrünthali, Castellani and Chalmers[2] (Table I), but it may be differentiated from these by its inability to produce gas in dextrin, raffinose and sorbitol.

The name Escherichia emphysemata is suggested.

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LABORATORY METHODS

A MODIFICATION OF THE McLEAN-VAN SLYKE METHOD FOR THE ESTIMATION OF CHLORIDES IN BLOOD*

By M. I. Hanna, B.A., Toronto, Canada

IN 1915 McLean and Van Slyke[1] introduced an iodometric technic for the determination of chlorides in small amounts of body fluids. Van Slyke and Donleavy[2] and Austin and Van Slyke[3] simplified the earlier method of protein precipitations so that accurate determinations of chlorides in plasma and whole blood could be made. Gettler[4] and later Short and Gellis[5] have modified this method in order to make it applicable to blood filtrates made by the Folin-Wu tungstic acid method. Though all of these methods are sufficiently accurate and satisfactory for estimation of chlorides in the laboratory, the technical procedures require too much time and too much material to be entirely suitable for clinical work. The following modification of the McLean-Van Slyke method has been found to fit well into the Folin-Wu system of blood analysis without an undue waste of time, which may be an important feature, particularly when dealing with cases of intestinal paresis. Further, the same blood filtrate which is required for other determinations on the patient may be used for estimating the chloride content of the blood.

METHOD

One part of oxalated blood or plasma is diluted with seven parts of water, and the pipette is washed with the diluted blood and drained twice. One part of 10 per cent sodium tungstate and one part of 2/3 N H_2SO_4 is then added as in the original Folin-Wu method. The mixture is vigorously shaken for a few minutes and filtered through a No. 40 Whatman filter paper into a dry flask. To 10 c.c. of filtrate are added 10 c.c. of distilled water and 5 c.c. of the M/29.25 silver nitrate solution (see Solutions). Allow to stand five minutes, then filter once through two small No. 40 Whatman filter papers folded together. A few drops are poured down the sextupled side of the filter and allowed to soak into the paper, the remainder is then poured into the filter. To 10 c.c. of filtrate, add 2 c.c. of starch citrate nitrite solution and titrate to the first permanent blue tint with M/73.1 potassium iodide solution, using a 5 c.c. standardized burette with a capillary tip dropping from 50 to 60 drops per c.c.

10.15 – double the titration value = mg. NaCl per c.c. of original blood.

EXPERIMENTAL

Several standard solutions of hydrochloric acid were made up by Hulett and Bonner's method and checked against alkali. They agreed satisfactorily.

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Samples of standard HCl were then analyzed for chloride content by the Austin-Van Slyke method and by the above modification. The results are shown in Table I.

In Table II are recorded a series of determinations on whole blood by the Austin-Van Slyke method and by the proposed modification. The maximum difference in titration values in the two methods is equivalent to 10 mg. of NaCl per 100 c.c. of blood; the maximal difference between all duplicates is equivalent to 6 mg. per 100 c.c. of blood.

TABLE I

CHLORIDE AS NaCl IN STANDARD HCl SOLUTIONS

(Theoretical Content 584.6 Mg. Per 100 C.C.)

	AUSTIN-VAN SLYKE METHOD	MODIFIED METHOD
	found	found
1	590	580
2	592	582

TABLE II

THE CHLORIDE CONTENT OF BLOOD BY THE AUSTIN-VAN SLYKE METHOD AND BY THE PROPOSED MODIFICATION

(As Mg. NaCl Per 100 C.C. of Blood)

SPECIMEN	AUSTIN-VAN SLYKE METHOD	PROPOSED METHOD	PERCENTAGE DIFFERENCE
1	510 513	505 505	−1.3
2	515 518	510 511	−1.2
3	495 495	489 495	−0.6
4	485 485	489 485	+0.4
5	485 490	495 495	+1.6
6	515 515	515 519	+0.4
7	495 495	495 495	0
8	490 495	499 499	+1.4
9	515 515	519 515	+0.4
10	535 535	535 539	+0.4
11	525 525	525 525	0
12	515 512	515 515	+0.3

DISCUSSION

The modified method gives results which are shown to be comparable with those obtained with the Austin-Van Slyke method. Satisfactory results are also obtained with the method in estimating chloride of plasma, pleural and ascitic fluids, urine, cerebrospinal fluid, and gastric juice, employing appropriate dilutions where necessary. With urine containing no albumin the preliminary precipitation with tungstic acid is omitted. The principal value of the method, however, lies in the small amount of blood filtrate required, the small number of operations, and the speed with which the determination can be performed. If blood filtrate is limited in amount, a single determination may be made, using half the quantities recommended here, and it is even possible with care to obtain satisfactory results on much smaller quantities of blood, though the additional trouble required will seldom make this a desirable procedure. Particular attention should be paid to four points. The reagents used must be chloride-free and the glassware carefully recalibrated. After addition of the silver nitrate to the diluted blood filtrate, the mixture must stand five minutes or more to permit of diffusion of occluded silver nitrate, and lastly, the double filter paper used should be Whatman No. 40. Doubtless other brands and qualities of filter paper may be found satisfactory, but thus far among those tested this particular kind has been the only one which combined rapid filtration with complete retention of the precipitated silver chloride on the first filtration. As has previously been pointed out by others in connection with other determinations on blood filtrates, the use of an excessive quantity of oxalate as an anticoagulant is to be avoided.

SUMMARY

A modification of the methods devised by Van Slyke and his collaborators for the estimation of chloride in blood, which is applicable to Folin-Wu tungstic acid filtrates, is here described. The method is shown to be accurate within 1 per cent, or of the same order of accuracy as the original method. The advantage of the modification lies in the smaller amount of time, of work, and of blood filtrate required for the determination.

SOLUTIONS

M/29.25 Silver Nitrate Solution (1)

Pure fused $AgNO_3$	5.812 gm.
HNO_3 Sp.G. 1.42	250 c.c.
Water to	1000 c.c.

M/73.1 Potassium Iodide Solution (2)

Pure KI	2.27 gm. per liter

Two and four-tenths gm. KI are diluted to a liter and used to titrate 5 c.c. of the silver nitrate solution with 5 c.c. of starch citrate nitrite solution and 5 c.c. of water. The strength of the solution is then adjusted so that exactly 12.65 c.c. of the KI solution is required. (0.15 c.c. is used for obtaining the end-point, 12.50 c.c. to precipitate the silver.)

Starch Citrate Nitrite Solution (1)

Sodium citrate $Na_3C_6H_5O_7$, 5½ H_2O	446 gm.
Sodium nitrite	20 gm.
Soluble starch	2.5 gm.

Dissolve the starch in 500 c.c. of water by boiling for several minutes; add the citrate and nitrate and heat until all is dissolved. Filter through cotton; wash the filter with hot water, and when cold, make up to 1000 c.c.

This solution is satisfactory for at least six weeks. When it is required infrequently, Short and Gellis'[5] solution has some advantages. The same amounts of citrate and nitrite are dissolved in 800 c.c. of water and made up to 1,350 c.c. The starch is made up weekly by boiling in 100 c.c. of water and diluting when cold to 150 c.c. The two solutions are mixed before use in the proportion of 1 to 9. Three c.c. of the mixture replaces 2 c.c. of the McLean Van Slyke starch citrate nitrite solution.

I wish to thank Dr. W. R. Campbell, who suggested this work, for his constant interest and help.

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A SIMPLIFIED TECHNIC FOR STUDYING THE SECRETION OF LYMPH*

By Lester R. Dragstedt, Ph.D., M.D., and Carl A. Dragstedt, Ph.D., M.D.,
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THE method in general use for studying the formation and flow of lymph in class or laboratory courses in physiology consists in collecting the lymph by means of a cannula placed in the thoracic duct. A meal containing large amounts of fat given several hours before the experiment greatly facilitates finding the duct, but even under these favorable conditions the operator often experiences considerable difficulty in differentiating the duct from strands of fat in the neighborhood, especially if the field is to any extent obscured by hemorrhage. This is entirely obviated, and the duct can be made to stand out conspicuously if to the meal previously given is added a small amount of fat(we have used butter) which has been saturated with some fat dye. Sudan III answers the purpose admirably. This method may also be of advantage in studying the course of the intestinal lymphatics, as after feeding the dye they stand out in marked contrast to the strands of fat in the mesentery and along the blood vessels.

*From the Department of Surgery, The University of Chicago, and the Department of Physiology and Pharmacology, Northwestern University Medical School.
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A SIMPLE ARTIFICIAL RESPIRATION APPARATUS*

By E. J. Van Liere, M.S., M.D.,† and R. S. Allen, B.S., M.S., Chicago, Ill.

AN APPARATUS for the administration of artificial respiration was devised by Dr. A. L. Tatum by using an air-driven windshield wiper of automobiles. The windshield wiper which was used is manufactured and patented by Folberth, Cleveland, Ohio. Dr. Tatum did not publish a description of his apparatus. It is a simplified adaptation of the Becker apparatus.

This instrument, however, was not very adjustable, as the outflow of air which corresponds to the inspiratory phase of respiration was considerably longer than the phase corresponding to the expiration. Furthermore, there was practically no pause between inspiration and expiration so that the lungs did not have an opportunity to collapse thoroughly. We have made an attempt to overcome some of these difficulties.

The windshield wiper proper will not be described in this paper, because it may readily be purchased on the open market. As far as it is known, it operates on the same principle as the ordinary positive pressure air-driven windshield wiper. Only those parts which are essential for its modification for the use of administration of artificial respiration will be described.

The only changes made in the mechanism were: first, a metal shaft *S* (Fig. 1) which operates the valve *E* was made to replace the original one. This shaft was made 3⅛ inches in length and ⅝ of an inch in diameter. It is attached to the stopcock of valve *E* as shown in Fig. 1 and in the photograph (Fig. 5). It is so adjusted on the rocking-gear inside the cylinder of the air-driven apparatus that it is allowed to rotate only 135 degrees—this is important in regard to the regulation of the outflow of air, corresponding to inspiration. Incidentally, this adjustment is very easily made. The only other change made in the air-driven mechanism is that an ordinary needle-valve (V_1, Fig. 1) is inserted in an aperture, already present in the apparatus.

Fig. 1 shows the arrangement necessary for the conversion of this type of instrument for artificial respiration. Fig. 4 in the diagram is the connecting unit which joins the cylinder with the other portion of the apparatus, that is, *C′* is inserted at the point *C* into the cylinder and *D′* is inserted into *D*. Dr. Tatum found that the diameter of the metal tubes, *A* and *B*, was important, as it is essential to distribute the proper amount of air to the cylinder of the air-driven apparatus and also to allow enough air for the animal. The diameter of this tubing is ¼ of an inch.

Compressed air from the pipe line in the laboratory enters at *A* (Fig. 4) and goes into pipe *B*, here a part of the air goes to *B′* and a part to *B″*. The

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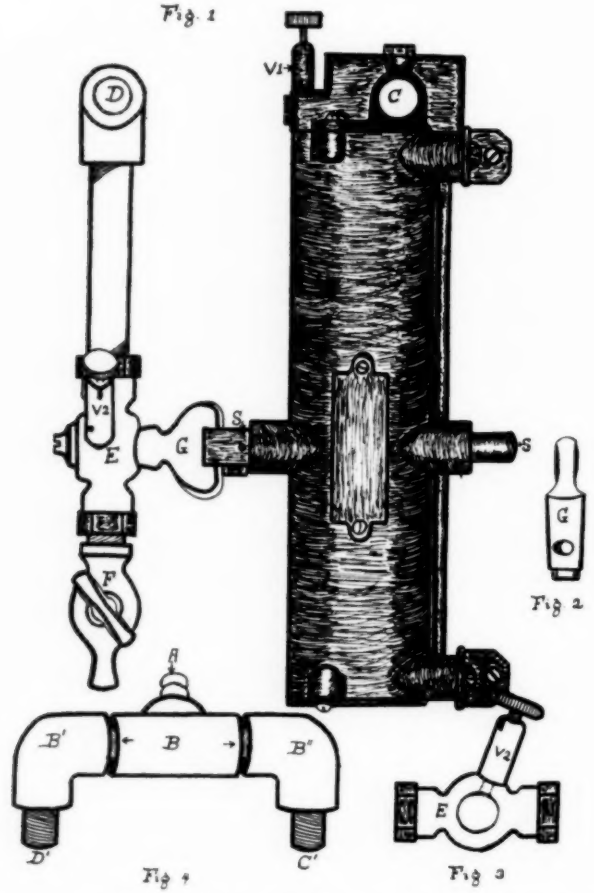
air which goes in the former direction is used by the animal; the air going in the latter direction is used to operate the air-driven mechanism. As explained above, the mechanism rotates the shaft *S* (Fig. 1) which in turn operates the valve *E*, which is merely a petcock valve. This valve, however, has a modification. The hole passing through the stopcock *G* is enlarged as shown in Fig. 2 by the shaded portion. Only the upper side is made larger; the hole in the lower part is not changed.



Figs. 1 to 4.—Diagram of artificial respiration apparatus. (Reduced one-half.)

Valve *E* has one more modification as shown in Fig. 3. A hole is drilled a bit off center on the upper surface of the petcock valve *E*, so as to coincide with the elliptical half of the hole made in the stopcock *G* of this valve. A needle-valve (or another petcock valve) is placed over this drilled hole as shown in the diagram. By changing the size of this aperture, the ratio of inspiration to expiration may be altered. It was found that when the needle-valve V_2 is closed, the ratio of inspiration to expiration is 1 to 1; when it is widely opened, the ratio is 1 to 5. By appropriate adjustment of the valve, the intermediate ratios may be obtained. Incidentally, this arrangement also makes it possible

to operate the mechanism with less air, so that it may be used for very small animals or in cases where only a small amount of air is desired.

The volume of air delivered at the outlet is controlled by the stopcock valve *F* (Fig. 1); the volume may also be varied by the amount of air allowed to enter at *A* (Fig. 4). The rapidity which the stopcock *G* of valve *E* (Fig. 1) is operated is controlled by the needle-valve V_1 on the cylinder, which has been described above. It may also be said that the rapidity with which this stopcock is turned in a given time is dependent to some extent on the air pressure

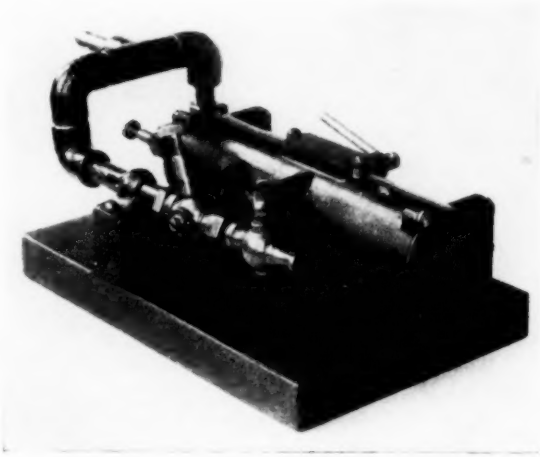


Fig. 5.—Artificial respiration apparatus.

entering at *A*, but this can be compensated for by resetting the valve V_1. The relation of inspiration to expiration is controlled by the needle-valve V_2 as previously described. In order to allow the lungs to collapse fully, it is well to insert a T-tube between the tracheal cannula and the outlet valve *F*.

To summarize: An apparatus is described in which the rate of respiration may be controlled; the volume of air at each inspiration may be controlled, and the ratio of inspiration to expiration may be controlled. It was found that this instrument is admirably adapted for small animals, but it may even be used for large dogs weighing from 20 to 25 kilos. This simple air interrupter, moreover, may be used for purposes other than the administration of artificial respiration. It is not complicated, so that it does not easily get out of order and lastly, it is quite inexpensive.

SIMPLIFIED APPARATUS AND TECHNIC FOR THE DETERMINATION OF THE ICTERUS INDEX*

By Russell C. Pigford, M.D., New Orleans

THE Meulengracht icterus index has proved a valuable adjunct in diagnostic and prognostic procedures. The technic, while comparatively simple to the average laboratory worker, offers disadvantages which tend to discourage the procedure as a routine laboratory test. The chief objections are: (1) many practitioners are not equipped with the Duboseq colorimeter, (2) in some instances accurate weighing of the small quantity of the potassium dichromate for the standard cannot be accomplished, (3) at least 5 c.c. of serum are required for the Duboseq apparatus, and (4) in sera with a high index repeated dilutions are necessary for the readings.

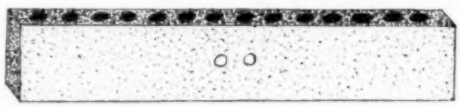


Fig. 1.—Front view showing small holes.

Fig. 2.—Back view showing large holes and ground glass back. *A*, tube for unknown. *B*, standard tube.

In the modified technic, standards of potassium dichromate solution of varying strengths are prepared and sealed in small test tubes. The strengths vary from 1:100, with an index value of 100 to 1:10,000, with a value of 1. The serial dilutions are readily made from a 1 per cent solution, acidified with a few drops of concentrated sulphuric acid. These standards are used for matching against the unknown serum in a manner similar to the technic of the phenolsulphonephthalein test.

Apparatus.—A wooden block ¾ of an inch wide, 2 inches high, and 12 inches long is required. With a 7/16-inch bit 14 holes, equally spaced, are bored to a depth of 1½ inches. From the side two holes are bored opposite holes numbers 7 and 8 (the middle holes) and communicating with these holes. Care must be exercised in boring the side holes as they are not to pass all the way through, but merely far enough to communicate with the holes from above. A piece of ground glass is placed across the two side holes. This is to act as a background for the matching. From the opposite side of the block two small holes (¼-inch bit) are bored so that they communicate with holes numbers 7 and 8 from above. By using the smaller holes in front, a greater con-

*From the Department of Medicine, Tulane University of Louisiana.
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trast is to be had in making the readings. The two central holes thus made act as a comparison box for the readings. One of the remaining 12 holes is to contain the empty test tube for the unknown serum. The other 11 are to hold the various graduated tubes of standards.

Twelve test tubes 9 mm. (inside diameter) by 45 mm., 2 c.c. capacity are used as containers. One is kept for the unknown. The other 11 are to contain the various strengths of the dichromate solution. After placing the solutions in the tubes, they are labeled and are sealed with melted paraffin, sealing wax, or by heat.

The dilutions found most satisfactory, with their index values, are given. They are :

Dilution	*Index Value*
1:10,000	1
1: 5,000	2
1: 3,500	3
1: 2,000	5
1: 1,500	6.7
1: 1,000	10
1: 666	15
1: 500	20
1: 400	25
1: 200	50
1: 100	100

To make the reading, 3 to 5 c.c. of blood is collected by venepuncture, allowed to clot, and after thorough centrifugalization, 20 drops of serum are placed in the empty test tube. Oxalated plasma may be used in place of the serum. This is then matched in the box against the various standards. If the index is greater than 100, proper dilutions can readily be made by adding an equal or double quantity of water and multiplying the reading by 2 or 3. Clear serum is to be desired, although slight clouding, as occasionally encountered in sera collected after a heavy meal, will not interfere with the reading. It must be remembered that very slight hemolysis will invalidate the reading.

The normal range of the icterus index is from 3 to 6. Latent jaundice ranges from 6 to 15. Above 15 the icterus is visible in the skin and mucous membranes. Values below 3 are encountered in the secondary anemias, while pernicious anemia will fall in the range of latent jaundice. The icterus index is of value in cases of obstructive jaundice. In these cases, the relief of the obstruction will be demonstrable by means of this test before other signs are present. A series of indices in cases of doubtful malignancy will sometimes give valuable diagnostic information. In the cases of malignancy the index will remain constantly above 100 while the benign liver will show a much greater fluctuation in the values obtained.

The method here described has the following advantages:

1. The equipment is inexpensive and can be made in the laboratory.
2. Determinations are rapidly made.
3. Only 1 to 1.5 c.c. of serum is required.
4. Calculations for dilutions are seldom necessary.
5. The readings obtained are sufficiently accurate for practical purposes.

A NEW ANIMAL BOARD*

By I. Forest Huddleson, D.V.M., and E. R. Carlson, D.V.M.

IN WORKING with laboratory animals, a prerequisite is an efficient means of restraint. We have constructed an animal board which is serving as a means for restraining animals much more conveniently than any other which we have used. It is constructed in such a manner that it may be used for holding rabbits, guinea pigs, or rats.

A detailed description and photographs of the board are presented in the hope that it will appeal to others.

The board proper (Fig. 1) is made of oak 1 inch in thickness, 11 inches wide, and 34 inches long. It is mounted on four legs 3 inches high. A

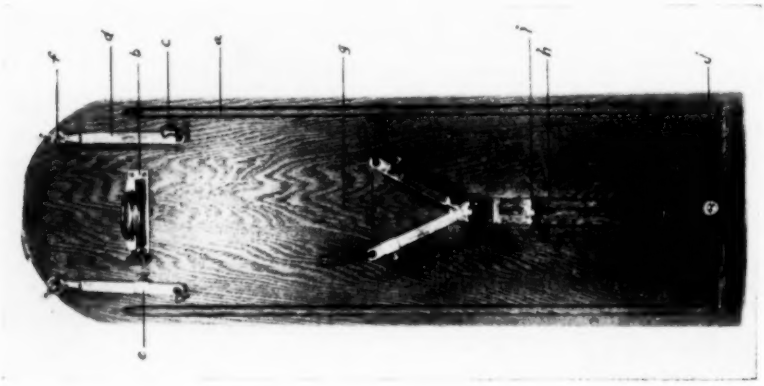

Fig. 1.

shallow groove (*a*) is cut near the edge of the top surface for collecting the excreta or blood which escapes during an operation.

Near the center of the top of the board (*b*) is located a half circle metal clamp for holding the head and neck. The ends of the open ring of the clamp pass downward into openings on either side of the fixed piece. The extended ends of the ring-piece are held in position by means of a set-screw situated on one side of the fixed piece of the clamp.

The anterior extremities are held in position by means of slip loops (*c*) made of stout cord attached to the ends of the metal arms (*d*). The size of the loop is determined by the position of the set-screw piece (*e*) which slides back and forth on the metal arm. The metal arm, which may be moved outward or inward is operated on the ratchet set-screw (*f*) principle. The pos-

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terior extremities are held in position by loops and metal arms similar to the one just described. (See Fig. 2.) The distance between the end of the arms is controlled from a central point (*g*) by a set-screw ratchet. The distance between the two sets of arms is controlled by the extension piece (*h*) which is

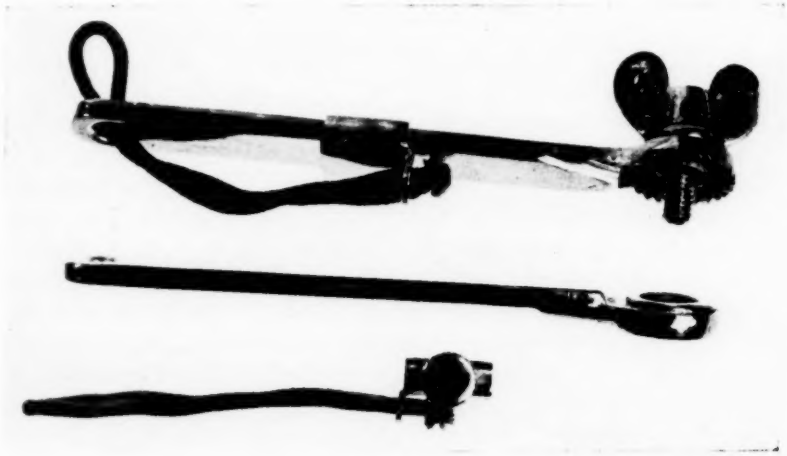

Fig. 2.

Fig. 3.

held in position by a set-screw (*i*). If one desires to make a further extension, the set-screw and extension may be moved back to (*j*) or the ends of the extension may be reversed without moving the set-screw from the position shown in the figure.

THE ESTIMATION OF RED CELL FRAGILITY
AN HEMATOCRIT METHOD*

By Herbert Silvette, Richmond, Virginia

VARIOUS substances have been employed to determine the resistance or the fragility of red blood cells. Of these, hypotonic solutions in varying strengths have been used with more or less satisfaction. The methods in use are those of Smith and Brown,[1] Karsner and Pearce,[2] Hill,[3] and Giffin and Sanford,[4] or some slight modification of these, such as that of Fontaine.[5] They are similar in that all use hypotonic solutions of sodium chloride as a means of estimating corpusele resistance. Simmel[9] employs a solution which is isotonic with blood, has the same P_H value, and the same percentage of the various inorganic salts. This solution, when used in four hypotonic dilutions, is the basis of a quantitative fragility test.

It has also been shown that red cells have a constant figure of resistance towards saponin and sapotoxin hemolysis. Saponin has been used by McNeil,[6] and by Bigland[7] as a means of determining red cell resistance, while sapotoxin was employed by Neilson and Wheelon[8] for the same purpose.

The hematocrit may be used to determine the fragility of red blood cells. Van Allen,[10] while working out experimentally the underlying principles governing the hematocrit, pointed out that "with a decrease of the concentration of the surrounding fluid, the erythrocytes increase in size until the limit of distensibility is reached, when the volume of the cells drop almost immediately to zero." With the use of several hematocrit pipettes and a series of hypotonic solutions of sodium oxalate of different concentrations, a method for the estimation of red cell fragility becomes both feasible and practicable. Such a method was suggested by Hamburger[12] in 1912, but there is no evidence of its present use.

For rabbit's blood it has been found[10] that the limit of distensibility of the red blood cells is indicated quite sharply at 0.72 per cent sodium oxalate. For human blood, the greatest volume of cells, that is, the limit of distensibility, occurs when a 0.6 per cent sodium oxalate solution is used. Invariably there is hemolysis, complete or incomplete, when a 0.5 per cent solution is employed.

It was decided, therefore, to limit the hypotonic solutions in the test to four: 0.5, 0.6, 0.7, and 0.8 per cent. The 0.5 solution would allow for any probable increase in the resistance of an unknown blood sample, while the range of 0.7 to 0.8 per cent would take care of any probable change in the direction of increased fragility.

These four hypotonic solutions should be made up as accurately as possible. To this end, about five hundred cubic centimeters of each strength

*From Pathological Laboratory, Johnston-Willis Hospital, Richmond, Va.
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should be prepared at a time, anhydrous sodium oxalate being used, weighed out on an analytical balance, and dissolved in distilled water in volumetric flasks. The solutions keep well when the containers are tightly corked, but in order to prevent the growth of molds, a few cubic centimeters of toluene should be added to each bottle. In view of the fact that a large quantity of each strength is made up at a time, and the concentration of sodium oxalate in the various solutions differs by a relatively large degree, it should be easy to secure constant results even when solutions made up at different times are used.

Four hematocrit pipettes[10, 11] are used, together with an equal number of appropriate sealing devices. Rubber bands may be used with perfect satisfaction, or the spring sealing device of van Allen.[13] An efficient and inexpensive sealing band, especially suitable to this method, is shown in Fig. 1. It consists of a cork attached to a rubber band by means of a small wire staple or pin. There is a hole half way through the cork into which the

Fig. 1.—An efficient sealing band for hematocrit pipettes.

upper extremity of the pipette is fitted; also the cork is marked with the strength of the diluting fluid with which it is to be used. It is very important to mark each pipette or sealing band with the concentration of the diluent in order to minimize the chance of error due to confusing the filled pipettes, and also to facilitate reading and recording the results.

With the four diluting fluids, the pipettes, and the sealing bands ready, the test is begun. A rather deep puncture is made in the patient's finger or ear, and the first drop of blood appearing is discarded. As a fresh quantity appears, it is drawn up into the bore of the hematocrit until the blood column is even with the top of the scale. Blood adhering to the outside of the pipette is wiped away, and a quantity of the 0.5 per cent diluting fluid is drawn up until the chamber of the hematocrit is about half filled. The sealing band is now attached, first to the graduated end of the pipette, and then to the bulb end. The three other tubes are now filled and sealed in the same manner, using the more concentrated solutions of sodium oxalate in turn. After a little practice, it becomes easy to fill the four tubes from a

TABLE I

RESULTS OF FRAGILITY TESTS ON 50 NORMAL SUBJECTS

MALE					FEMALE				
	HEMATOCRIT READINGS					HEMATOCRIT READINGS			
	PER CENT SODIUM OXALATE					PER CENT SODIUM OXALATE			
	0.5	0.6	0.7	0.8		0.5	0.6	0.7	0.8
S	72	83	67	62	R	56	64	57	50
O	57	73	63	53	S	42	54	54	47
C	54	70	58	55	S	44	66	52	50
C	72	67	58	50*	R	44	64	50	49
W	49	70	52	52	W	50	56	54	43
F	54	61	55	55	G	42	54	50	45
H	45	75	59	49	F	56	64	57	45
W	33	61	56	52	E	57	61	58	52
S	45	71	61	55	W	60	63	54	49
E	56	62	49	48	T	50	57	51	48
C	61	72	59	49	V	56	65	55	46
B	74	80	63	63	G	62	65	51	45
C	47	74	61	54	F	44	66	56	53
F	51	68	53	50	A	41	58	51	45
F	61	71	50	48	C	63	55	44	44*
G	41	65	50	49	M	50	56	51	47
D	55	70	59	51	P	41	64	56	45
H	63	81	70	60	P	22	66	55	45
M	47	66	44	44	H	12	57	43	41
J	53	79	68	56	B	23	63	58	55
S	41	64	60	51	M	24	59	53	45
T	59	80	61	59	W	6	63	57	49
L	60	69	62	56	D	32	46	40	37
R	46	59	50	43	K	23	58	54	46
F	52	77	55	54	K	4	71	58	44
Av.	54	76	58	53	Av.	40	60	53	47

*Atypical normal curves, showing increased resistance.

single puncture. A little pressure some distance away from the stab is permissible, indeed necessary, but should the blood coagulate because of too slow collecting and handling, another prick must be made.

The hematocrit pipettes are now placed in the centrifuge, the even number of tubes doing away with any special counterbalancing. Centrifugalization is carried out at 2700 revolutions per minute for fifteen minutes, these having been found by van Allen to be the optimum speed and duration. It is imperative that all pipettes in a single test be centrifuged for the same length of time and at the same rate of speed in order to obtain comparative readings. After removing the pipettes from the centrifuge, the results are read and recorded.

Results are expressed and interpreted by means of a fragility curve, drawn by using the hematocrit readings as ordinates and the concentration of sodium oxalate as abscissas.

In a series of fifty healthy, normal subjects, both male and female (Table I), the greatest cell volume in each case occurred when 0.6 per cent sodium oxalate was used as the diluent. Also, in every instance some hemolysis took place using the 0.5 per cent solution, thus giving a well-defined peak for the normal fragility curve at 0.6 per cent concentration of sodium oxalate (Fig. 2). Where the peak of the curve is found to be at 0.5, that is, the greatest volume of cells obtained is with the 0.5 per cent solution, increased corpuscle resistance is indicated and the type of curve may be referred to as a

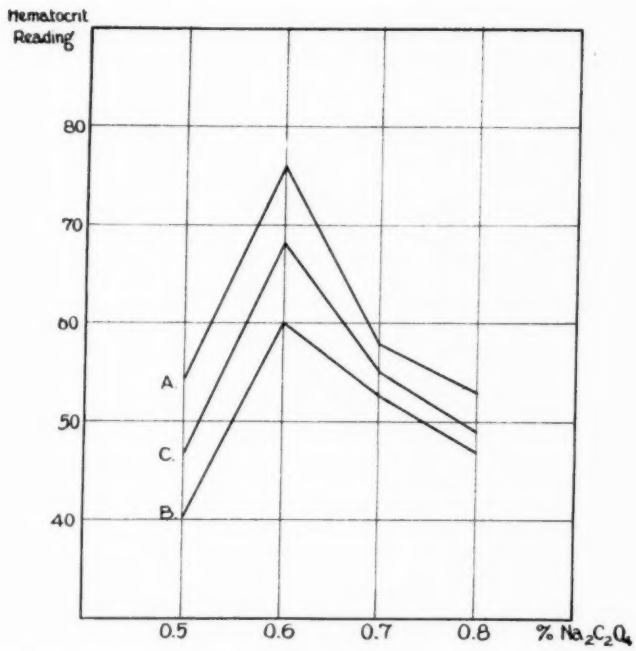

Fig. 2.—Average normal fragility curves. *A*, average of the fragility curves of 25 normal men; *B*, average of the fragility curves of 25 normal women; *C*, normal fragility curve, average of both sexes.

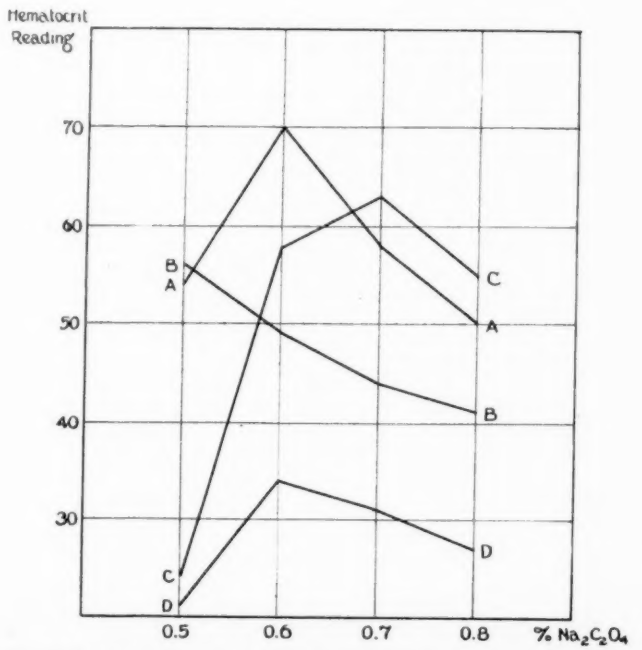

Fig. 3.—Typical fragility curves. *A*, normal curve, for comparison; *B*, curve of increased resistance, from a case of obstructive jaundice; *C*, curve of increased fragility, from a case of childhood nonobstructive jaundice; *D*, normal curve, from a case of severe secondary anemia.

"curve of increased resistance" (Fig. 3-*B*). Where the peak of the curve is to the right of 0.6, either at 0.7 or at 0.8, the result may be called a "curve of increased fragility" (Fig. 3-*C*). The degree of either resistance or fragility of the red cells is directly proportional to the degree of displacement, either to the left or to the right, of the peak of the curve in question compared with that of the normal curve at 0.6 per cent.

It is also possible, by means of the angle of the line forming the left side of the peak of the curve, to interpret the results to a much finer degree than the placement of the peaks would indicate. To illustrate, Fig. 4-*A* is a normal curve, and while curves *B* and *C* in the same figure are also to be considered normal, it is noticeable that *B* shows a tendency toward increased

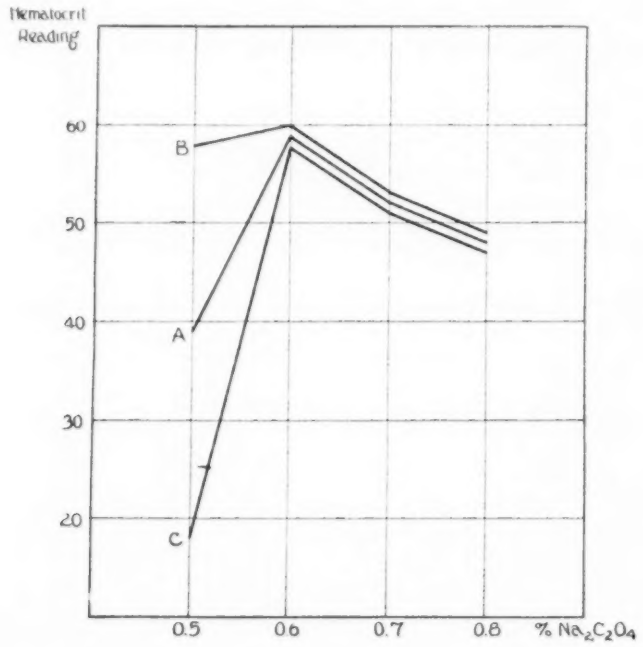

Fig. 4.—Normal fragility curves. *A*, average normal curve; *B*, normal curve with a tendency toward increased corpuscle resistance; *C*, normal curve with tendency toward increased corpuscle fragility.

cell resistance, and *C* toward increased corpuscle fragility. Pathologic curves may be read to the same degree as normal ones, and in general, it will be noted that the more nearly vertical the line forming the left side of the peak, the more fragile are the cells. Conversely, the more nearly horizontal that line is, the more resistant the cells. Since the peak of the curve of increased resistance is not complete on the left side, this statement will not apply. In that case, a falling line between 0.5 and 0.6 would denote a greater degree of corpuscle resistance, and a line approaching the horizontal a somewhat lesser degree. It is then possible to interpret the results of a hematocrit fragility test with as much, if not greater, accuracy as the results indicated by the two-figure index of initial and complete hemolysis.

TABLE II

RESULTS OF FRAGILITY TESTS ON A SERIES OF PATHOLOGIC CASES

NAME	DIAGNOSIS	HEMATOCRIT READINGS PER CENT SODIUM OXALATE				CURVE
		0.5	0.6	0.7	0.8	
B.	Secondary anemia	18	32	30	28	N.
C.	" "	28	30	27	25	N.
D.	" "	33	35	32	29	N.
C.	" "	11	34	30	25	N.
W.	Obstructive jaundice	56	49	44	41	I.R.
J.	" "	57	53	47	44	I.R.
B.	Nonobstructive jaundice	23	58	63	55	I.F.
R.	Ruptured gall bladder; bile in peritoneal cavity	32	47	45	45	N.

N, normal curve; I.R., curve of increased resistance; I.F., curve of increased fragility.

The results of the hematocrit method for the estimation of red cell fragility give substantially the same results as do any of the methods using hypotonic saline. A normal curve is obtained in anemia (Fig. 3-*D*), and curves of increased resistance in obstructive jaundice (Fig. 3-*B*). In a single instance of childhood nonobstructive jaundice, a curve of increased fragility was obtained (Fig. 3-*C*). In practically every normal subject (48 out of 50), a normal result was obtained; the abnormal curves both pointing to a slight increased resistance of the cells in question.

It is not necessary, as it is when using venous blood and hypotonic saline, to include a normal control in the test. The diluting fluids may be prepared accurately enough to give comparative results, and on the whole, the operation of the hematocrit is so standardized that the limit of error in a carefully performed test is negligible.

CONCLUSIONS

The hematocrit method for the estimation of corpuscle fragility has the following advantages over the other methods in use at the present time:

1. Capillary blood, obtained from either the finger or ear, is used, thus eliminating venipuncture.

2. Fragility tests may be made without difficulty on infants and children. This usually has been inconvenient due to the difficulty of obtaining several cubic centimeters of blood in such cases.

3. Only four hypotonic solutions are used in the test, in place of the dozen or more where saline is used. Furthermore, the sodium oxalate solutions need not be made up freshly and are stable.

4. The test is completed within thirty minutes. The time element is thus reduced considerably.

5. A normal control is unnecessary, due to standardization of materials and technic.

6. The interpretation of results is easier and more accurate than is the case using a two-figure index. Results are expressed as a "fragility curve" comparable to a curve of a "glucose tolerance test."

7. Results with the hematocrit method are constant.

SUMMARY

An hematocrit method for the estimation of red cell fragility has been described, using four hypotonic (0.5, 0.6, 0.7, 0.8 per cent) solutions of sodium oxalate, four hematocrit pipettes, and capillary blood. Results are expressed in a fragility curve. This method possesses several advantages over the methods using venous blood and hypotonic saline. Results with the test are constant and normal in healthy, normal subjects, and vary as do the results of the other methods in disease.

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THE BUNSEN VALVE IN BLOOD-UREA DETERMINATIONS*

BY F. P. BROOKS, PH.D., CHAPEL HILL, N. C.

EXPERIENCE has shown that the Folin-Wu method for the determination of blood urea presents many technical difficulties. That these difficulties have not yet been satisfactorily overcome is evident from the many modifications appearing in the journals and from the attempts being made to find methods offering less difficulties of manipulation. The chief troubles with the method seem to have been two in number. First, the urease solutions have caused foaming, and second, the distillation has been attended by frequent aspiration of the receiving acid back into the distilling tube, due to the sudden cooling of the distilling tube by air currents or variations in the heat of the burner.

Of the many modifications suggested, it appears to me that the one proposed by Johnson[1] is the simplest and best solution of the problem. This method eliminates the back aspiration by closing the end of the delivery tube with a flutter valve and allows the operator to center his attention upon the prevention of foaming. In considering this method it occurred to me that the difficulty of finding flutter valves of the proper size might be obviated by

*From the Laboratory of Physiological Chemistry, School of Medicine, University of North Carolina, Chapel Hill, N. C.
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[1]Johnson, S. Lloyd: JOUR. LAB. AND CLIN. MED., 1924, ix, 860.

the use of a device, namely, the Bunsen valve, which can be easily and quickly made by anyone with a small piece of thin-walled tubing, a piece of glass rod, and a razor blade.

The Bunsen valve is an instrument, already familiar to the chemist, consisting of a piece of rubber tubing preferably of about three-sixteenths inch bore and with walls from one-sixteenth to one-eighth inch thick. One end of the tube is closed by a piece[1] of glass rod. One or more slits are made lengthwise in the sides of the tube close to the closed end. These should be made with a sharp razor blade and should be from one-quarter to half an inch in length. This constitutes the Bunsen valve. When placed on the end of a bent glass tube, as originally used by Folin-Wu, it affords a satisfactory flutter valve. When the contents of the distillation tube are boiled, the air and steam escape through the slits in the valve. Whenever any back pressure develops, these slits close tightly and prevent the back suction of the acid

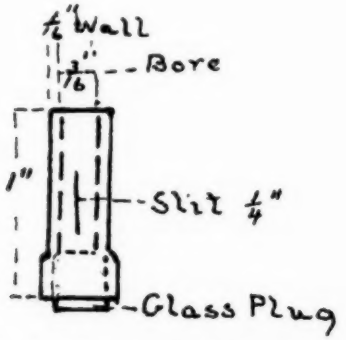

Fig. 1.—Bunsen valve. (Actual size.)

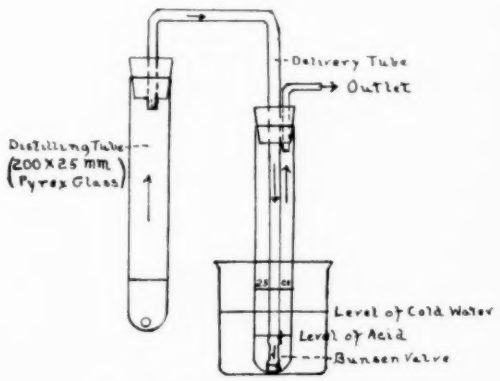

Fig. 2.—Folin-Wu urea distilling apparatus (according to Johnson) with the Bunsen valve attachment. (⅛ actual size.)

from the receiving tube. This device has the advantage over the flutter valve devised by Johnson of being quickly constructed from materials easily accessible to the average operator.

This device solves the problem of back aspiration and allows the heating of the distillation tube to be varied with the foaming. However, it has been my experience that this removal and exclusion of air has little or no effect upon the foaming. As a solution for this problem I have found that a small piece of fixing paraffin (M.P. 47-49), inserted in the distillation tube before heating is commenced, helps to overcome this problem. Before the contents of the tube reach the boiling point, this becomes liquid, and by surfacing the walls of the tube helps greatly to keep down foaming. Paraffin oil has been found to distill over and to interfere with "Nesslerization" by causing clouding. Very little of this paraffin goes over and when the receiver and its contents are cooled the paraffin solidifies and rises to the top. "Nesslerization" of such a solution gives a perfectly clear solution which can be compared in

the colorimeter just as satisfactorily as two standard solutions. There is at most only a trace of ammonia in the paraffin, and this can be taken into consideration by running blank determinations.

Numerous determinations run on standard urea solutions using these two improvements in technic show recovery within 5 per cent of theoretical values and these variations are probably due to errors incidental to the use of the colorimeter.

SUMMARY

The use of the simple Bunsen valve is suggested as a substitute for the flutter valve devised by Johnson in blood-urea distillations by the Folin-Wu method. The use of fixing paraffin as an antifoamant is found to be satisfactory.

AN AID IN THE ESTIMATION OF BLOOD CALCIUM*

By E. M. Watson, M.D., M.Sc., London, Canada

THE physiologic and pathologic significance of the inorganic elements of the blood is gaining recognition. Not the least important in this regard is the blood calcium. Unfortunately, the quantitative estimation of this substance presents greater technical difficulties than most other clinical laboratory procedures. Therefore, any device facilitating its determination would seem salutary. An apparatus is described which has been found useful in the routine estimations of serum calcium.

For accurate results with the volumetric method of determining the blood calcium, as, for example, by the Kramer-Tisdall technic,[1] certain necessary conditions must be fulfilled. These, as outlined by Clark,[2] are several. Among other things, the titration of the oxalate solution with potassium permanganate should be carried out at a certain temperature, namely, 75° C. Also, there is the necessity for keeping the solution well stirred during the course of the titration. The accompanying illustration shows the result of an attempt to provide for these two requisites by a simple means.

In Fig. 1, *A* is a micro-burette containing N/100 potassium permanganate. *B* is a 15 c.c. graduated centrifuge tube in which the calcium oxalate has been precipitated and subsequently dissolved in normal sulphuric acid. Behind this tube and also immersed in the water-bath, is a piece of flash opal glass (*c*); behind this again is a small electric light bulb, within a sealed test tube. *D* is the handle of the stirring contrivance, an up and down movement of which serves to mix, simultaneously, the liquids within and without the cen-

*From the Laboratory of Pathological Chemistry, University of Western Ontario Medical School.

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trifuge tube. The light, which may be controlled by the switch (*E*), assists in deciding the end-point of the titration.

The apparatus described in this note was assembled by Mr. A. Barber, my technical assistant.

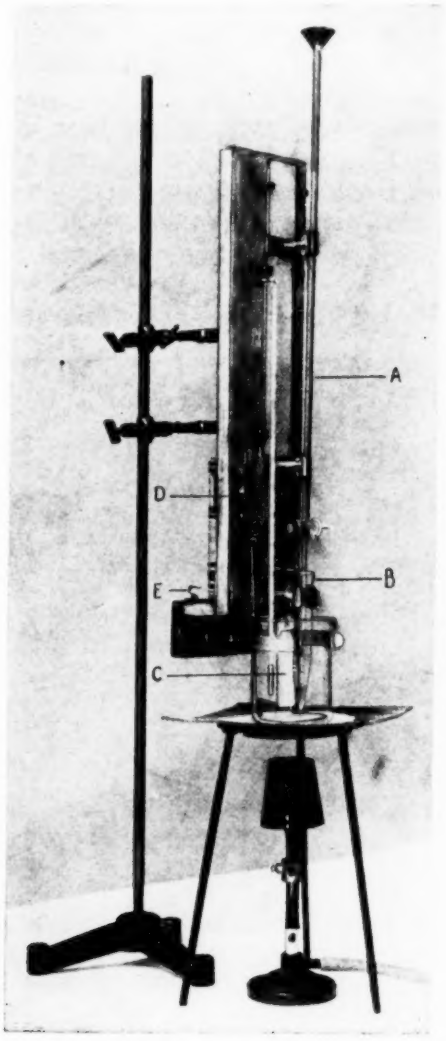

Fig. 1.

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NOTES ON LABORATORY TECHNIC*

By Thomas B. Magath, M.D., Rochester, Minnesota

BLOOD CULTURES

While a great number of technical methods have been described for making blood cultures, none seems to fulfill the needs of everybody since each individual has certain problems which are not common to others. At the Mayo Clinic a number of methods of making blood cultures have been tried and the present one, which has now been in use for more than seven years and followed in more than 3500 cases, has proved satisfactory. The conditions which had to be fulfilled are as follows: (1) the method must be applicable

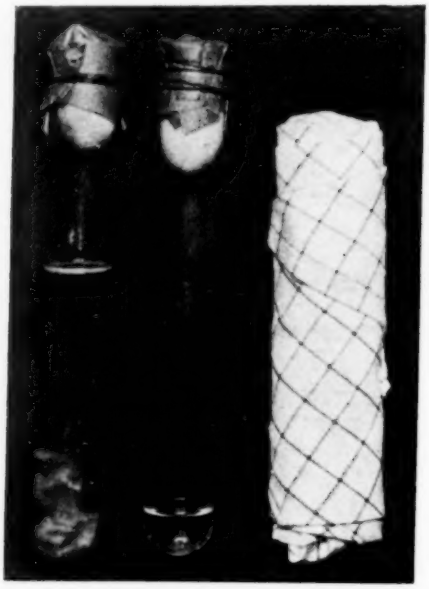

Fig. 1.—Blood-culture outfit: the towel contains a baked syringe and its needles; the test tubes contain respectively glucose-brain broth and sodium citrate solution.

in all types of septicemia; (2) reports must be rendered with reasonable promptness; (3) there must be an opportunity to decide whether or not a positive culture is due to contamination; (4) a large number of cultures have to be taken; (5) the patient may be ambulatory and the blood be drawn in the laboratory or, (6) the blood may have to be drawn at the bedside in private homes or in hospitals.

The following equipment is necessary and is prepared as follows:

1. A 20 c.c. Luer syringe and two needles, one No. 19 and one No. 21, are wrapped in a towel and baked at 170° C. for an hour.

*Section on Clinical Pathology, Mayo Clinic.
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2. Two test tubes (2.5 by 20 cm.) are used, one containing 5 c.c. of a 1 per cent solution of sodium citrate, the other containing dextrose-brain broth. The brain broth is put into the tube to a height of 15 cm. and both tubes are plugged with gauze and cotton stoppers, the tops being wrapped with brown paper which is secured with a rubber band (Fig. 1). Both tubes are autoclaved. This paper covering does away with the necessity of flaming the tubes at the time of drawing the blood.

Blood is drawn from a suitable vein in the arm after the skin has been prepared by thorough washing with alcohol; other preparation has been found unnecessary and of no advantage. From 15 to 20 c.c. of blood is secured in a dry syringe, from 3 to 5 c.c. being placed immediately in the brain-broth tube and the rest in the solution of citrate. The tubes are brought to the laboratory, the brain-broth tube being immediately placed in the incubator and

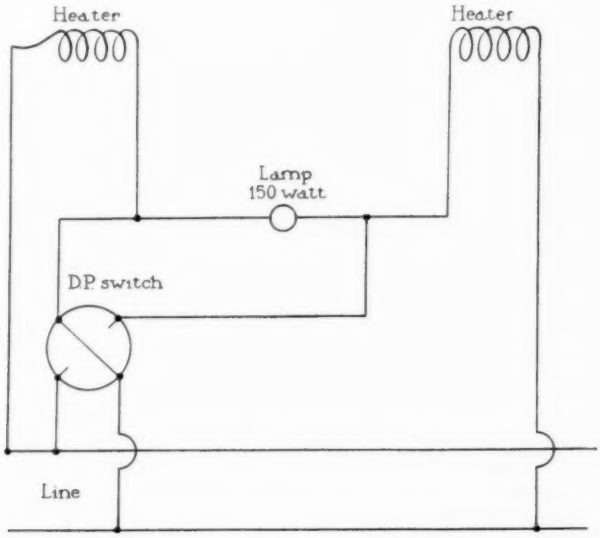

Fig. 2.—Microscope lamp showing slots for screens.

blood agar plates being made from the citrated blood. Hormone agar is used as a base, and four plates are poured, the first containing 0.5 c.c. of blood, the second 1 c.c. of blood, the third 2 c.c., and the fourth 3 c.c. The plates and the tubes are examined at the end of twenty-four hours; if negative they are put back in the incubator for another twenty-four hours, the report being made at the end of forty-eight hours. The brain-broth tube is then set aside in the incubator and examined at weekly intervals for three weeks. Recently we have been employing a moist chamber in the incubator and holding the plates prepared from certain types of cases for a week. By distributing the blood on plates in this fashion and comparing them with the brain-broth tube, it is possible in most cases to recognize contamination; moreover, the method permits of a quantitative report on the number of colonies for each cubic centimeter of blood. The brain-broth tube has enough liquid in it together with the brain tissue to bring about reduction in oxygen tension,

thereby increasing the possibility of growing certain streptococci in Rosenow's opinion. The method has proved satisfactory in culturing hemolytic and nonhemolytic streptococci, colon bacilli, typhoid bacilli, anthrax bacilli, pneumococci and influenza bacilli. It is a simple, direct method, with a minimum of labor for the technician and gives reliable results.

A SIMPLE MICROSCOPE LAMP

With the increasing use of binocular microscopes and the developing of laboratories in the heart of business sections in cities, artificial light has come to be an important problem to the microscopist. Most of the microscope lamps on the market require special bulbs that are short-lived and which do not give sufficient light; the large types are entirely too expensive to be used in laboratories requiring large numbers of microscope lamps. Following is a description of a lamp which cost $1.50 and which could be made in large

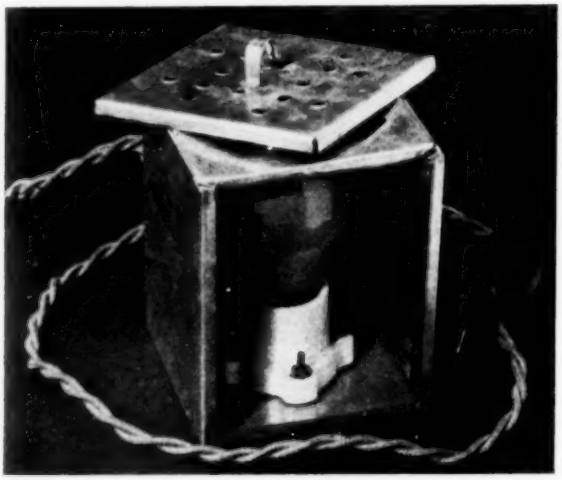

Fig. 3.—When the switch is in the "off" position, the heaters are in series and the temperature is about 46° C.; when in the "on" position, the heaters are in parallel and the water boils. The plug is withdrawn to disconnect.

quantities for less and is more satisfactory than any light yet used. It is made of polished tin; its dimensions are 10 by 10 by 12.5 cm. In the bottom an ordinary porcelain bulb-socket is secured by two screws. The front is made with two grooves into which are fitted glass screens. The source of light is an ordinary frosted Mazda 40 or 60 watt bulb. The top is movable and is ventilated, as well as the back. Electric light wires protected by the usual insulation are connected with the socket (Fig. 2). The screens are prepared by staining a developed photographic plate. For examining unstained material, such as fresh stool, a screen stained with a dilute solution of Orange G makes an excellent light. Daylight glass can be purchased, making a white light for use with stained material, and any shade of blue may be obtained by staining a developed photographic plate with methylene blue. These screens last for years and give excellent results.

TABULATION

TEST	AMOUNT OF BLOOD, C.C.	DISPOSITION OF BLOOD
Wassermann	4	In a Wassermann tube.
Coagulation time	1*	Rinse syringe and Wassermann tube with normal solution of sodium chloride, test every 30 seconds.
Calcium coagulation time	1*	As coagulation test but after rinsing tube, add 6 capillary drops of 0.5 per cent calcium chloride to the tube.
Prothrombin	4*	Add 4 c.c. blood to graduating centrifuge tube in which has been placed 0.5 c.c. of 1 per cent potassium oxalate solution. Invert tube to mix.
Retractility of clot	1*	In a Wassermann tube.
Fragility	2	Add one drop of blood to each of 12 tubes containing sodium chloride in varying concentrations.
Grouping	4	Add 3 drops to a Wassermann tube half full of 2 per cent sodium citrate solution; the remainder in a dry tube.
Widal and other agglutination tests	4	In a Wassermann tube.
Methemoglobin and sulphemoglobin	3	Add to 5 c.c. of distilled water; shake.
Serum bilirubin	8	Rinse syringe and centrifuge tube with normal solution of sodium chloride first.
Sugar	6	In large oxalate tube (1 c.c. of 3 per cent potassium oxalate heated to dryness); shake well.
Urea and creatinine	8	As for sugar.
Uric acid	8	As for sugar.
Inorganic phosphorus	12	As for sugar.
Lipoid phosphorus	8	As for sugar.
Chlorides	6	As for sugar.
Calcium and magnesium	8	Centrifuge tube and syringes must be rinsed thoroughly in distilled water.
Carbon dioxide combining-power	6	In tube containing dry oxalate and mineral oil; shake.
Cholesterol	3*	Add to bottle containing 25 c.c. ether and 75 c.c. alcohol; shake.
Blood culture	15	2 to 5 c.c. in large brain-broth tube and remainder in large test tube containing 5 c.c. 1 per cent sodium citrate sterilized. Use baked syringe.
Volume index	10*	Graduated centrifuge tube in which is placed 2 c.c. of 1.6 per cent sodium oxalate.

*Exact measurement.

A METHOD OF KEEPING MELTED AGAR

For more than four years we have used two Universal water heaters, capacity about one quart each, for keeping agar melted in the laboratory. The two heaters are so wired with a switch and a lamp that, in one position of the switch, water is boiled in both containers and, at another position of the switch, the temperature is reduced to about 46° C. which keeps the agar melted. The diagram of the wiring is given in Fig. 3, where it can be seen that the light bulb and the heating elements are used for resistance. During the four years, the only repairs have been replacement of the lamp once and

of the small fuses once each. The apparatus is in constant use, burning all day, and melted agar at the right temperature is always on hand.

DIRECTIONS FOR TAKING BLOOD SPECIMENS FOR VARIOUS TESTS

The accompanying tabulation has been found useful when placed in the bleeding rooms where blood is being taken for various tests; it is also mounted in the tray which is used in collecting samples in the hospitals. It leaves no doubt in the technician's mind as to the exact method of taking blood specimens for the various tests which may be performed in the laboratories. A slight modification of the table might be useful in almost any laboratory.

A METHOD FOR HANDLING LARGE NUMBERS OF SLIDES IN STAINING FOR THE BACILLUS TUBERCULOSIS

Two possible methods of staining for bacillus tuberculosis in routine laboratories are available; one consists of staining each slide separately, the other of staining slides in bulk. The objections to staining in bulk have usually been that more stain is used than necessary and that the constant heating of the stain causes precipitation; on the other hand, the objection to the slide-staining method is that the staining is more laborious and time-consuming and is usually messy. The tendency of the slides to become scorched together with the constant necessity of watching them, makes the method not an ideal one. With a smaller vat, less stain can be used, and if the procedure is properly carried out, the results are extremely satisfactory. The staining vat which we have used is a Vollrath refrigerator pan 15 by 15 by 7.5 cm. It is kept on an electric hot-plate and is heated and kept at a temperature of 80° C. while the staining is in process. The decolorizing and counterstaining reagents are kept in glass refrigerator dishes purchased at a novelty store at a cost of 10 cents each. These dishes have covers and measure 12 by 12 by 5.5 cm. In our laboratory three methods of staining are carried on simultaneously. The first consists of heating with carbolfuchsin for five minutes, decolorizing in 10 per cent nitric acid in water and then in 95 per cent alcohol and counterstaining with methylene blue. The second varies from the first in that 5 per cent sulphuric acid in 95 per cent alcohol is used as a decolorizing reagent. The third is the Schultze-Tigges method which consists of staining for one minute with carbolfuchsin, destaining with fresh 10 per cent sodium sulphite and counterstaining with picric acid. The method I have described is suitable for handling a large number of slides by the three methods simultaneously. The important feature is the carrier for the slides (Fig. 4). It is made of aluminum 2.5 mm. thick; the frame is 6.25 cm. wide and 10 cm. long. An aluminum guard made of 1.5 mm. material is attached to each side and bent parallel to the side. From one guard to the other measures 7.8 cm. The aluminum sides that hold the slides are slotted for thirty slides. Occasionally one can find on the market an aluminum comb which has the teeth set at the exact distance to hold the slides. The ends of the carrier are suitably shaped to carry a wire spring handle (not shown in the illustration) and are marked with numbers serially to indicate the batch. The slides are dropped in in order.

after being numbered with a diamond pencil, and are stained in bulk. If 100 slides are stained each day, the carbolfuchsin should be changed once a week; the other reagents will last for at least a week and the counterstaining reagents longer. The results have been extremely satisfactory for a long time, and this method eliminates much of the drudgery of routine staining by the single-slide method. The slide carriers were originally designed by Whitman for use in tissue-staining and improved upon by Lundquist but, since the aluminum withstood the acid used in staining bacilli of tuberculosis, the method has been developed for that use.

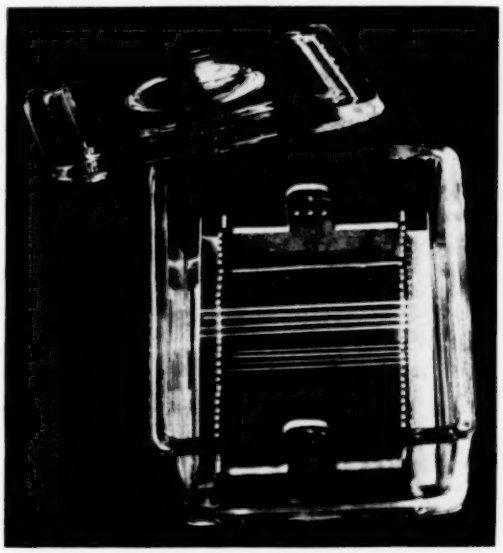

Fig. 4.—Slide-carrier in staining-vessel.

METHOD FOR MAKING TISSUE CULTURES

The most common way of taking cultures of tissues, those that have been removed surgically or at the necropsy table, is to sear the surface with a hot spatula. This is often unsatisfactory and fails to give uncontaminated cultures. A solution to the problem is offered by the use of the Lenk Improved Automatic Torch. This gives a direct, very hot pencil flame. It operates with alcohol and is ready almost immediately after lighting; the instrument is simple, positive and inexpensive. The flame is also suitable for flaming the mouths of test tubes and for pipettes. The percentage of contaminations will drop immediately upon its use.

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COLLOIDAL GOLD SOLUTION*

The Preparation of Gold Solutions and Their Titration With Permanent H-Ion Concentration Standards

By Vincent Christina and Clara S. Green, New York City

INTRODUCTION

ONE of the most important items in the Lange gold test for the differentiation of cerebrospinal syphilis is the necessary neutrality of the gold solution employed. It has been found extremely difficult to prepare strictly neutral solutions with consistently satisfactory results. We suggest a slightly modified mode of preparation and a simple and reliable method of titration and correction of the colloidal gold solution.

The methods generally accepted as the best for obtaining bright red, completely clear and stable solutions, are those of Zsigmondy,[1] without oxalic acid, and of Miller and his coworkers,[2] with oxalic acid. We have found the use of oxalic acid unnecessary. The investigations of Zsigmondy and his coworkers show that if it is desired to obtain a fine-grained, bright red, clear, and stable solution, the velocity of formation of nuclei must be taken into consideration. The importance of the velocity of formation of the nuclei has been shown in the formaldehyde reduction method. If reduction takes place in less than a minute, we obtain bright red, clear solutions due to the large number of particles; while if the reduction of the gold is retarded, we obtain highly fluorescent and unstable solutions ranging in color from blue to purple. Zsigmondy has also found that the presence of ammonia reduces the velocity of formation of nuclei, and that CO_2 interferes with the production of red, fine-grained solutions. Therefore, every precaution must be taken to insure their absence.

In the formaldehyde method we start with acid gold chloride and a slight excess of K_2CO_3, since it is known that reduction takes place best in a slightly alkaline medium. The following reaction[3] occurs:

1. $HAuCl_4 + 2K_2CO_3 + H_2O = Au(OH)_3 + 2CO_2 + 4KCl$
2. $2Au(OH)_3 + K_2CO_3 = 2KAuO_2 + 3H_2O + CO_2$
3. $2KAuO_2 + 3HCOOH + K_2CO_3 = 2Au + 3HCOOK + H_2O + KHCO_3$

Since the CO_2 formed interferes with the production of fine-grained solutions, the heating is continued after adding K_2CO_3 until all of the gas is removed.

To overcome the interference of dissolved ammonia and CO_2 in water, conductivity water is used in this laboratory. The titration of gold solutions with alizarin, the method most frequently employed, is unsatisfactory for sev-

*From the Bio-chemical Department of the New York State Psychiatric Institute, Ward's Island.

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eral reasons. Alizarin as an indicator has too wide a range, and the color change after the addition of this indicator is not sharp enough with small increases of acid or alkali to warrant its use.

The hydrogen electrode method is indubitably the most accurate, but it is time-consuming and too expensive for clinical laboratories. The colorimetric method of determining P_H value is simple and rapid, provided that correct buffer and indicator solutions are at hand. However, a certain amount of difficulty lies in the fact that these solutions are unstable, and their repeated preparation is difficult and consumes much time.

We have found Taub's[4] permanent standards in a slightly modified form satisfactory. Our permanent standards prepared according to this method were checked against the Lovibond tintometer, and Hynson, Westcott and Dunning organic standards. The gold solution which was standardized with our inorganic standards was also checked against Hynson, Westcott and Dunning organic standards and the hydrogen electrode.

PROCEDURE

Glassware.—Use new Jena glassware, which has been thoroughly washed with soap and hot water. Then soak in hot dichromate cleansing mixture overnight, rinse thoroughly under tap water, next with distilled, and just before use with conductivity water.

Conductivity Water.—To every liter of distilled water (from a Stokes still) add 1 gm. of chromic acid and distill in an all-glass distilling apparatus, avoiding use of rubber stoppers and connections. To every liter of water distilled over chromic acid, add 1 gm. of barium hydrate. This may now be kept as a stock supply to be redistilled immediately before use.

Reagents.—Merck's gold chloride acid yellow—1 per cent solution. Merck's potassium carbonate C.P.—2 per cent solution. Merck's neutral formaldehyde solution—1 to 40 dilution. These reagents are made up with fresh conductivity water.

Preparation of Colloidal Gold.—Heat 1 liter of fresh conductivity water to 60° C.; at this temperature add 10 c.c. of 1 per cent gold chloride solution and 8 c.c. of 2 per cent potassium carbonate solution. Continue the heating until the temperature reaches 92° to 95° C., then remove the flame and add 5 c.c. of formaldehyde solution, 1 to 40 dilution. Stir briskly until a full, bright red color develops.

The necessity of brisk and even stirring must be emphasized, otherwise reduction will not be uniform. After the solution has cooled to room temperature, preferably after standing overnight, the P_H of the gold solution may be determined.

TITRATIONS

The permanent inorganic standards are prepared, with slight modification, according to Taub. The following solutions are necessary for a P_H range of 6.6 to 7.4:

N/2 cobalt chloride . . . . 59.497 gm. $CoCl_2 .6H_2O$ per liter of 1 per cent HCl.

N/2 ferric chloride . . . . 45.054 gm. $FeCl_3$.$6H_2O$ per liter of 1 per cent HCl.

The indicator is prepared as follows: Dissolve 1 gm. of phenol red in 52 c.c. of neutral 95 per cent alcohol, add 28.5 c.c. of N/100 NaOH and sufficient conductivity water to bring the volume up to 250 c.c. Store the indicator in a Pyrex bottle.

MATCHING BLENDS

Table I indicates the amounts of the chlorides necessary to give the correct colors for the standards.

TABLE I

P_H	CO (C.C.)	FE (C.C.)	CONDUCTIVITY H_2O (C.C.)
6.6	1.8	6.5	11.7
6.7*	2.1	5.95	11.95
6.8	2.4	5.4	12.2
6.9*	2.95	4.15	12.9
7.0	3.5	2.9	13.6
7.1*	4.25	2.1	13.65
7.2	5.0	1.3	13.7
7.3*	6.1	0.8	13.1
7.4	7.2	0.3	12.5

*Our own additions.

The solutions are placed in test tubes of uniform length, diameter, and thickness. They are then sealed into ampoule form and sterilized in the autoclave at 15 pounds pressure for fifteen minutes to insure complete sterility. The tubes are allowed to cool overnight.

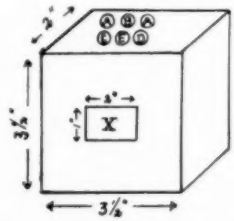

Fig. 1.—*X* equals perforation with frosted glass attached in rear.

A comparator block (Fig. 1) is used to make the P_H determination. In making determinations the test tubes should be of the same diameter and thickness as the uniform tubes used for standards.

To 10 c.c. of the gold solution, add 0.15 c.c. of indicator and mix well. Place this tube in Hole *E*. In Hole *B* place a tube with distilled H_2O. In Holes *A*, *A*, place tubes containing gold solution without indicator. Holes *C* and *D* are for the P_H standards. If the gold solution does not match the 7.0 P_H standard, then N/100 NaOH or N/100 HCl is added, as the case may require. If the solution shows a P_H below 7.0, alkali is used for the titration; if it is above 7.0, then acid is employed. After each addition of acid or alkali to the gold solution, the contents of the tube are thoroughly mixed by inverting the tube several times.

When the unknown gold solution is matched with the 7.0 standard, the necessary correction is made for the entire volume of gold on hand. It is a practice in this laboratory to make a P_H determination on our gold solutions immediately before use, as they have a tendency to become slightly alkaline after two weeks' standing.

After correction, we test the neutrality of the solution by adding 1.7 c.c. of 1 per cent NaCl to 5 c.c. of the colloidal gold. The gold will be completely precipitated in an hour. It must give a typical curve with a known paretic spinal fluid, and no reaction, or a red-blue change at the most in the first or second tube, with a negative spinal fluid.

SUMMARY

1. A slight modification of Zsigmondy's method of preparation of colloidal gold is described, wherein conductivity, instead of triply distilled water is used, in addition to an increased amount of alkali.

2. The titration of the solution is accomplished with phenol red as an indicator, and the P_H is determined with permanent inorganic standards.

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A NEW AUTOMATIC PIPETTE*

Incorporating a Mechanically Operated Two-Way Valve

By F. W. Hartman, M.D., Detroit, Mich.

IN THE large laboratory the problem of pipetting accurately measured quantities of fluids entails much monotonous yet exacting labor. This applies particularly in the set-up of the Wassermann test and in the filling of ampoules. A number of automatic pipettes have been proposed, notably those of Woodyatt, Lorenze, and Laird. As in all automatic pipettes these depend upon a two-way valve, and in each of those mentioned the valve is actuated by the flow of fluid through it or by the flow of fluid plus a spring. In the use of these pipettes it was found that they needed frequent adjustment, especially when reagents, such as ambocepter, antigen, and red-cell suspensions were used or when the speed of operation varied. The height of the supply bottle and delivery tip when modified also produced variation in the delivered amounts.

It occurred to us that valves actuated by the flow of fluids through them could not be expected to give uniform or accurate delivery under varying con-

*From the Laboratories of the Henry Ford Hospital.
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ditions, particularly when the motor speed was altered or when fluids tending to make the valves stick, as reagents containing serum or lipoids, were handled.

To avoid variations in the delivered amounts under varying operating conditions, it seemed essential to have the valve operated mechanically with

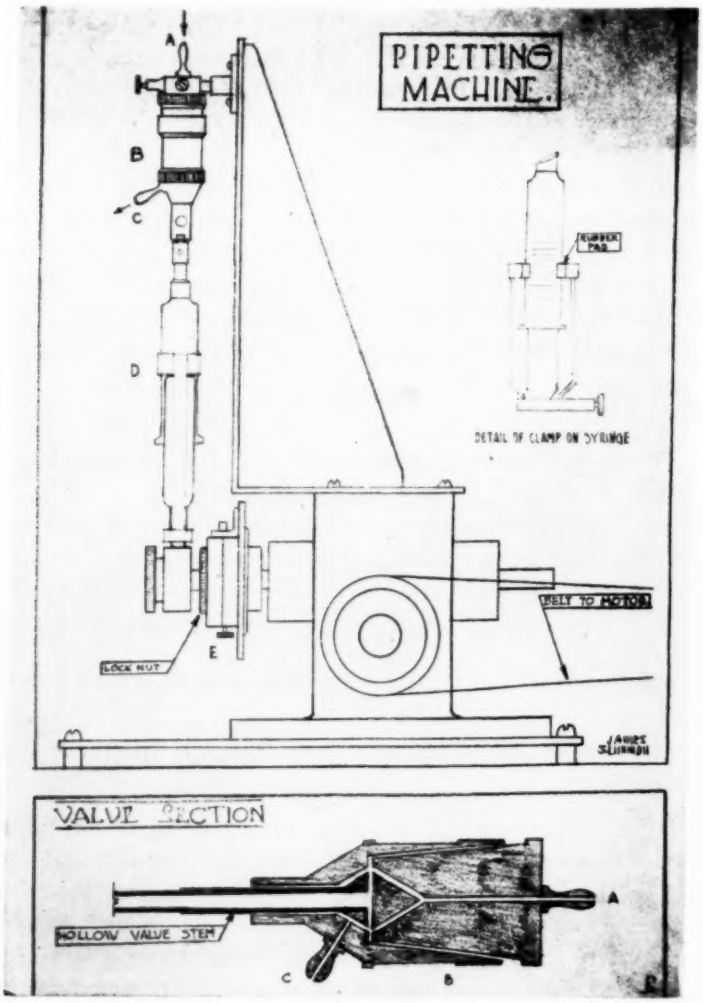

Fig. 1.

the action of the syringe plunger, thus making the valve independent of the speed and force of the fluid current.

An apparatus incorporating a mechanically operated valve which is still automatic was finally evolved. The principle depends upon the attachment of the valve core to the barrel of the syringe then actuating this barrel by friction either from the plunger or in combination with an outside friction pad.

A cross-section of the valve is shown in the lower illustration (Fig. 1). The valve seat block *B* is made in two parts which are held together by a

slip-lock, so that they may be readily separated and the valve seats cleaned. The valve core is a double cone with a stem extending from the block with an attachment at the end for a Luer-loc (Becton-Dickinson) syringe. From the sides of the cone, openings connect with the hollow valve stem. When the valve is assembled, as shown in the illustration, and the lower cone of the core is seated by pulling on the valve-stem, fluid may enter, flowing around the upper part of the cone and into the valve-stem through the openings in the sides of the core. If the cone is pushed against the upper valve-seat, the intake is securely blocked and fluid in the valve-stem may be forced out the lateral openings in the core around the lower cone and out at *C*.

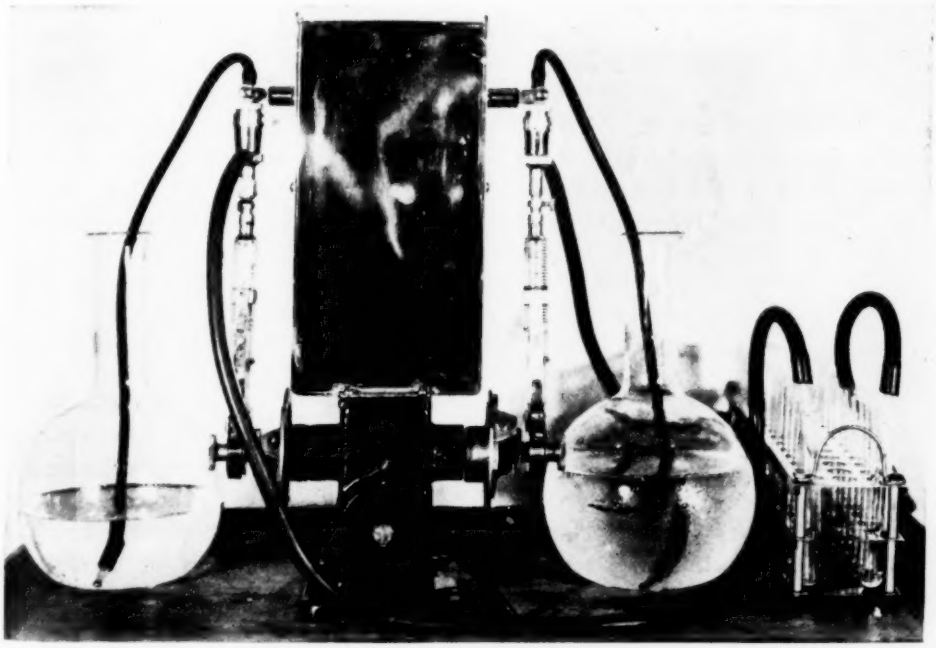

Fig. 2.

The valve attached to a Luer-loc syringe and the whole mounted with gear and adjustment is shown in the upper illustration (Fig. 1). The adjustment *E* controls the length of the piston stroke and is made with screw and lock-nut. This adjustment when used with a 5 c.c. syringe will allow delivery of from $\frac{1}{10}$ to 5 c.c. At slow speeds and with a 10 c.c. syringe the larger amounts may be delivered.

The barrel of the syringe and the attached valve core is actuated by the friction of the plunger with each upward or downward stroke, and this is supplemented by a clamp which is attached with the syringe piston to the drive shaft below. The upper portion of the clamp ends in two rubber friction pads which make readily adjustable friction against the syringe barrel.

The machine is driven by a one-eighth horse power motor, the speed of which should be adjustable. In operation, the fluid is taken up from a flask

on the table through a tube to *A*, into the syringe and out at *C*, being delivered through pressure tubing to test tubes or ampoules.

Smaller amounts, that is 1/10 to 1 c.c., may be delivered at rates of from 100 to 200 per minute. Larger amounts up to 5 c.c. may be delivered at rates of 50 to 100 per minute. With a still larger syringe and a larger valve 50 or 100 c.c. amounts may be handled rapidly. A double pipette is easily arranged to run with the same motor and gear (Fig. 2). With this double arrangement two operators are necessary, but twice the amount of pipetting may be accomplished. It will be noted that the syringe, valve, and tubing are quickly detachable, so that they may be sterilized and replaced without disturbing the adjustment.

As to accuracy, if the temperature is controlled, the variation is less than one-half of 1 per cent. Although checked many times a day with different solutions, the variation is found negligible. The apparatus was not designed for a pressure pump, but it will deliver accurately against a pressure of 300 mm. of mercury when the friction clamp is properly adjusted. Necessary adjustments under usual working conditions are made on an average of once a month, and these are usually after cleaning or replacement of syringes. When changing from one reagent to another, saline is washed through the apparatus for a period of five minutes. Care is taken to flush the machine for the same period before leaving it for the night. Cleaning is readily accomplished by detaching the syringe and disassembling the valve block. If there is an accumulation of material on the valve seats or core, this may be removed with dilute cleaning solution.

SUMMARY

A positive accurate automatic pipette incorporating a new mechanically operated two-way valve is presented. The use of the machine in Wassermann work gives greater accuracy than obtained by hand pipetting, saves much time, and eliminates most of the monotonous drudgery of putting in the salt solution and various reagents. Nearly two years operation in our own laboratories show repairs and replacements rarely necessary.

The mechanical perfection of this machine was made possible by Alfred Schmitt, instrument maker of the Henry Ford Hospital, who has built the original models.

The complete apparatus is manufactured by the Detroit Laboratory and Hospital Supply Company, 8019 Third Avenue, Detroit, Michigan.

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A NOTE ON THE REFLEX NATURE OF STRYCHNINE CONVULSIONS*

By Seymour J. Cohen, M.S., M.D., Chicago

THE fact that strychnine causes convulsions which are reflex in origin has been known for years, but the actual demonstration for class work has been quite unsatisfactory. As early as 1846, Meyer[1] showed that on cutting the posterior roots of all the spinal nerves in frogs and then injecting strychnine, no convulsions appeared. When the central end of the nerve was touched, marked convulsions occurred, thus showing that a sensory stimulation is necessary to initiate the convulsions. Hering[2] repeated this experiment, but put the strychnine directly on the cord. Again there were no convulsions unless the animal was severely shaken or the central stumps of the nerves were touched. These experiments are very difficult to demonstrate to the students in a laboratory.

Paulson,[3] in 1889, showed, by painting the skin of a frog with cocaine or chloretone, thereby paralyzing the sensory nerve ends, injection of strychnine was not followed by convulsions unless the animal was strongly stimulated. Meyer[1] used 30 per cent hydrocyanic acid to paralyze the nerve endings with the same results. These experiments also show that a sensory stimulation is necessary to initiate a strychnine convulsion. They are objectionable, however, because if too much cocaine is absorbed by the skin and reaches the circulation, it may accentuate these convulsions and defeat the point of the experiment; while too much chloretone may depress the spinal cord and consequently in itself abolish the convulsions. Hydrocyanic acid is much too dangerous to use in the laboratory.

All the above experiments are open to criticism, being either too difficult to perform in a classroom or else some of the drugs may be absorbed and modify the experiment.

A simple method of showing conclusively that the convulsions caused by strychnine are reflex in origin is as follows: In a decerebrated frog one sciatic nerve is exposed and cut. Then ½ mg. of strychnine is injected into the ventral lymph sac. The leg with the cut sciatic nerve is touched with a probe, no convulsions occur. The other leg with the nerve intact is touched, and is followed by marked convulsions throughout the body of the frog.

Inasmuch as the impulse cannot travel centrally in the cut sciatic nerve, no stimulus is received by the cord and hence no convulsions. This simple experiment can easily be demonstrated and shows quite conclusively the reflex nature of strychnine convulsions.

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[1]Meyer: Ztschr. f. Ration. Med., 1846, v, 257-261.
[2]Hering: Arch. f. d. ges. Physiol., 1893, liv, 614-636.
[3]Paulson: Arch. f. Exper. Path. u. Pharmakol., 1889, xxvi, 22.

*From the Laboratory of Pharmacology, University of Illinois College of Medicine, Chicago.

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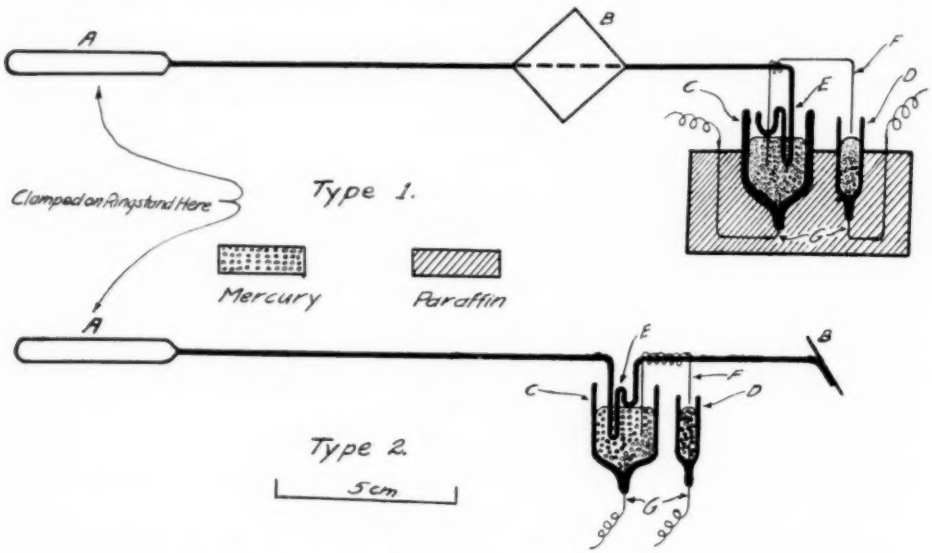
A SIMPLE DROP COUNTER*

By Edgar J. Poth, Ph.D., Baltimore, Md.

A SIMPLE, easily adjusted, and efficient device for counting drops is often needed in biologic work. In the accompanying illustration two types of such an arrangement are given.

A, in each case, is a glass rod or tube drawn out and bent as shown. *B*, in Type 1, is a cover slide made fast to the rod with beeswax, sealing wax, or DeKhotinsky cement. In the case of Type 2 only a part of a cover slide is used for *B* so as to keep the weight beyond *E* a minimum. *C* and *D* are two mercury cups with sealed-in platinum wires (*G*) and containing mercury. The wires (*G*) are leads to the usual electric signal system assembled so as to write on a



revolving drum. *F* is a platinum wire, fastened with beeswax or similar material, and serves to complete the electrical circuit at *D* whenever a drop falls on *B*.

The important part of this arrangement is at *E*. The buoyant action of the mercury on the longer shank of the rod, which is submerged, keeps the rod under stress and, due to the viscosity of the mercury, eliminates vibrations in the sidewise or horizontal directions. The shorter shank with its lower bend adjusted so as to rest just on the mercury surface arrests up and down or vertical vibrations.

The final adjustment is made by varying the height of the mercury in *C* and *D*. The result is an easily adjusted, rapidly acting, and fully damped device for counting drops, which at the same time is simple in construction and entirely reliable as to accuracy.

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NOTE ON THE VOLHARD-HARVEY METHOD FOR THE ESTIMATION OF CHLORIDES IN URINE*

By Willis H. Jeffery, Providence, Rhode Island

IN THE course of class work in the quantitative analysis of urine for chlorides, some difficulty has occasionally been encountered in the clear perception of the end-point in the Volhard-Harvey method. Such difficulty is usually found to be associated with a very concentrated twenty-four-hour sample, one in which the volume is six hundred cubic centimeters or less. Such a sample often has an amber color which tends to mask the appearance of the color of the ferric ammonium thiocyanate. In cases of this sort, it has been found helpful to dilute the five cubic centimeters to fifty or more cubic centimeters.

But a hindrance which cannot be ascribed to this source has recently been noted. A particular sample of urine of average volume and light straw color gave a brownish violet color when treated with the reagents employed in this method. Subsequently, it transpired that the color was due to the acidified indicator alone. Then it seemed of interest to learn whether other ferric salts would also give the color. When saturated ferric chloride solution was added to some of the sample, a purplish red color, unobscured by any precipitate, was obtained. The color was so like the false positive result of the Gerhardt test for aceto-acetic acid, that is commonly if not always observed with urines containing salicylates, that it was at once suspected that the subject had taken aspirin just prior to the collection of the sample. Upon inquiry it was learned that he had taken two aspirin tablets on the evening before the collection of the sample was started.

Further trials with aspirin on different subjects were made, and it became evident that the ingestion of one aspirin tablet was sufficient to give the above-mentioned color with the acidified indicator, over a period of twenty-four to thirty-six hours. In order to insure the observation of a sharp end-point, the employment of an extra dish containing an untitrated mixture, to serve as a control, together with the first rough titration, was found indispensable.

*From the Arnold Biological Laboratory of Brown University.
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AN IMPROVED TECHNIC FOR DEMONSTRATING EXPERIMENTAL JACKSONIAN EPILEPSY*

By Lester R. Dragstedt, Ph.D., M.D., and Carl A. Dragstedt, Ph.D., M.D., Chicago, Ill.

THE following is a modification of a technic in use in the Physiologic Laboratory of the University of Chicago for demonstrating the effect of an electric stimulation of the mortor cortex of experimental animals. The symptoms resemble those seen in Jacksonian epilepsy. Dogs are preferably used. The animal is anesthetized and the field prepared with careful aseptic precautions. A median incision is made over the vertex, and the skin is widely reflected. The temporal fascia and muscle is divided along its origin and peeled laterally from the bone. Two small holes, about two millimeters in diameter, are drilled through the calvarium over the motor area, preferably one hole on either side. Electrical binding posts which have been previously sterilized are screwed into the holes, the brass screws penetrating the dura. Slits are then made in the temporal muscle and skin over the posts to permit these to extend to the exterior. The incision is closed. When the animal recovers from the anesthetic, wires leading from the secondary of an inductorium may be connected with the binding posts extending into the motor cortex and direct electrical stimulation with varying strengths of current may be obtained. It is best not to attempt to keep the animal for more than a day or so as infection invariably occurs along the course of the electrodes.

A METHOD FOR STUDYING THE SECRETION OF URINE IN EXPERIMENTAL ANIMALS†

By Carl A. Dragstedt, Ph.D., M.D., and Lester R. Dragstedt, Ph.D., M.D., Chicago, Ill.

THE chief objections to the usual methods of studying the secretion of urine in animals both for experimental investigation and in laboratory exercises for didactic purposes are first, the trauma incident to the operative procedures required for placing the cannulae in the ureters, which will often inhibit the secretion of urine for a varying period, and second, the toxic effect of the anesthetic on the kidneys. The following method permits of a very accurate study of the urinary secretion in animals under practically normal conditions. During the course of an experimental investigation of the surgery of the urogenital tract it was found that dogs will survive the produc-

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tion of an artificial exstrophy of the bladder for long periods of time without developing kidney infections. These animals serve admirably for a study of the physiology of urinary secretion, the action of diuretics, and tests for kidney function. The operative procedure is not difficult. Females are selected for this work; a median incision is made just above the symphysis pubis, and the bladder is secured and separated from its peritoneal attachments. The urethra is divided and closed, the bladder opened, and the bladder wall trimmed away except for a small circular area which contains the orifices of the ureters. This area is stitched into the abdominal wall on closure of the incision. The wound heals readily, and animals prepared in this fashion can be kept in the laboratory indefinitely without requiring any special attention. It is possible to secure the secretion from either ureter separately by means of cannulae or from both by means of an inverted funnel strapped to the abdominal wall. The animal should be secured in a wooden frame during the collection of all samples.

Erratum

In the article by Blumberg, entitled "Pathology of Intestinal Tuberculosis," February, 1928, issue, the second sentence, eleventh paragraph, page 412, should read: The nonfermentative pasty, acholic, viscous stool, etc.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

TUBERCULIN: VI. Acid Hydrolysis of Tuberculin, Long, E. R. Am. Rev. Tubere., May, 1926, xiii, No. 5, p. 441.

A highly potent tuberculin prepared on synthetic medium was neutralized, and normal hydrochloric acid was added to 50 c.c. samples of it in such amounts that final concentrations of the acid were N/100, N/20, N/10, N/6, and N/3. These samples, with one which was neutral, were heated in the autoclave four hours at 120°. They were then neutralized with sodium hydrate, and to them salt and water were added in such amount that the final volume and salt content were the same in all. The first four samples (beginning with the one heated at neutrality) gave powerful skin reactions in a subject sensitive to tuberculin. A trace of reaction was given with the fifth sample and no reaction with the sixth. Trichloracetic acid gave a precipitate immediately with the first hour preparations, a trace of precipitate on standing in the fifth, and a smaller trace with the sixth. The trichloracetic acid precipitates were filtered off, and all the filtrates were saturated with ammonium sulphate. A precipitate developed in all tubes. The first four precipitates of this series had tuberculin activity, but not the last two.

TUBERCULIN: V. The Effect of Proteolytic Enzymes on Tuberculin Proteins and the Activity of Tuberculin, Seibert, F. B. Am. Rev. Tubere., May, 1926, xiii, No. 5, p. 393.

Whenever the whole protein molecule (noncoagulable water-soluble fraction) is attacked and changed to proteose and amino acids, as by the action of pepsin or trypsin with Na_2CO_3, there is a complete loss in activity of the protein, as determined by negative skin tests in tuberculous guinea pigs. The effect is a direct result of enzymic action, since boiling of the enzyme before mixing destroys its ability to bring about these results.

Trypsin, in neutral solution, attacks first proteose, with no loss in activity.

Erepsin, an enzyme which is not able to attack the whole protein molecule, but which is able to act upon the peptide bonds already free in the proteose fraction, does not cause any loss in activity.

These experiments indicate that the active substance of the water-soluble noncoagulable fraction used in this study is a whole protein molecule, or else it is so closely associated with this protein that its activity is lost when the whole protein molecule is disrupted.

TUBERCULIN: VII. The Evidence That the Active Principle Is a Protein, Long, E. R., and Seibert, F. B. Am. Rev. Tubere., May, 1926, xiii, No. 5, p. 448.

The active principle of tuberculin appears to be a whole protein. The experimental evidence for this is as follows: The active principle of untreated tuberculin is a colloidal substance which does not pass through animal and vegetable membranes, except in traces. A colloid giving all the usual protein tests may be precipitated by acids at its isoelectric point, P_H 4.0. This substance is highly active in the tuberculin test. Precipitation of protein is not complete by this method, however, and the filtrate, containing some protein, also retains tuberculin activity. Saturation of tuberculin solutions with ammonium-sulphate precipitates all detectable protein. The precipitate is the most highly active of all tuberculin preparations obtained. The filtrate contains no protein and shows no tuberculin activity whatever. The most specific reagents for protein at our disposal, proteolytic enzymes, lessen or destroy

tuberculin activity at the same time that they disrupt protein present. As whole protein decreases and protein cleavage products accumulate in solutions of tuberculin subjected to proteolytic enzyme action, tuberculin activity decreases or disappears. When whole protein disappears on acid hydrolysis activity is lost. As long, however, as whole protein is present activity remains.

There is evidence that a protein product of smaller molecular weight than "whole protein" may be active. Active principle in traces dialyzes. "Proteose" obtained by ammonium-sulphate precipitation, after "whole protein" has been precipitated by trichloracetic acid, has some activity. These results signify that activity is associated primarily with the whole-protein molecule, but persists in spite of a certain amount of disintegration of the molecule.

CARCINOMA: The Relation Between the Surface Tension of Blood Serum and Its Calcium Content in Carcinoma, Svehla, K. Compt. rend. Soc. de Biol., April 23, 1926, xciv, 980.

Surface tension was determined by Bang's torsion balance and expressed as dyn/cm. Calcium was determined by Kramer and Tisdall's method.

In carcinoma the average surface tension was 51-96 dyn/cm as compared to 63.67 dyn/cm in normal individuals.

The average calcium content in carcinoma was 0.185 mg. per cent as compared to 0.232 mg. per cent in normal individuals.

The lowering of the surface tension in carcinoma is in direct proportion to the lowering of its calcium content.

OZENA, BACTERIOLOGY: Bacillus Ozena Foetidae, Perez, and Bacillus Proteus in Ozena, Michailoff, A. Bull. Johns Hopkins Hosp., September, 1926, xxxix, No. 3, p. 158.

Ozena is a chronic disease of the nose characterized by a mucopurulent discharge, crusts, a specific fetid odor, and atrophy of the turbinate bones.

A bacteriologic study of twenty-eight cases. The bacillus described by Perez was isolated in only seven cases. A bacillus of the proteus group was also found with sufficient frequency to warrant study of its connection with the disease.

Bacteriologic and immunologic studies are reported in detail and the author's conclusions are as follows:

Although the cultural and fermentative properties are insufficient to identify Perez bacillus as atypical Proteus, we are justified in thinking that it constitutes a sub-group which has lost some of its fermentative properties, a frequent occurrence with the most typical proteus, and that B. ozawnae liquefaciens Shiga is a typical B. proteus vulgaris.

The agglutination and agglutinin absorption tests show Perez bacillus to be very closely related to B. proteus; Perez X is intermediate between the two species, having agglutinative properties as strong for Perez bacillus as for B. proteus. Each bacillus of this group has a serologic individuality more or less close to the Perez or proteus species. Of the fourteen bacilli, no two are identical. Agglutinin absorption shows clearly that the motile strains, B. ozaenae liquefaciens Shiga, and B. proteus vulgaris, as a rule, have their coagglutinative branches reduced or removed when absorption is done with the motile bacillus, and vice versa; they are more closely related to each other than to the nonmotile Perez species and can be identified as belonging to the same group as B. proteus.

Complement fixation shows that the two varieties, motile and nonmotile, have a very close relation. Some Perez and Proteus bacilli have identical properties as antigens against a given serum and this reaction appears to show closer relationship than the agglutination or agglutinin absorption test. There is no correlation between the complement-fixation and agglutination test.

The more specific flocculation reaction also shows the organisms to be very closely related, but only Perez II and Hofer's Perez bacilli have identical properties, while of these only the latter shows flocculation with B. proteus.

B. proteus, B. Perez, and B. ozaenae liquefaciens have identical pathologic properties. Each produces the four different types of infection: (a) toxico-septicemic congestive and hemorrhagic lesions; (b) chronic pyemic, mucosal, and endothelial exudative and proliferative lesions; (c) local exudative lesions; and (d) special necrotic lesions due to complex pathologic processes.

All the strains studied produce a toxin identical with that of Hofer's Perez bacillus and Proteus vulgaris.

Cross immunization shows that Perez bacillus and B. proteus have identical immunizing properties, protecting completely or producing sufficient protection to indicate their identity or very close relationship.

On the basis of the above conclusions it follows that the Perez bacillus is a member of the large group of Proteus bacilli.

The etiologic relation of the Proteus-Perez group to ozaenae fetida has been suggested by many workers because of the frequent finding of these organisms in the nasal discharge of such cases. The Proteus group exhibits an affinity for the blood vessels and mucosae, has pathogenic power, producing chronic necrotic lesions and nasal ulceration and discharge. We never produced green crusts or atrophy of the turbinate bones in the rabbit, and therefore we cannot conclude that in human beings B. proteus produces ozaena fetida, and that this bacillus acts as a primary etiologic agent. The frequency of its occurrence and the specific malodor found in the cultures show that B. proteus is involved in the pathogenesis of ozaena, and whether implanted primarily or secondarily, is the cause of the fetor, discharge and ulceration.

TYPHOID COLON GROUP MEDIA: A Combination of Bismuth and Sodium Sulphite Affording an Enrichment and Selective Medium for the Typhoid-Paratyphoid Groups of Bacteria, Wilson, W. J., and Blair, E. M. Jour. Path. and Bacteriol., July, 1926, xxix, 310.

To 100 c.c. of ordinary 3 per cent nutrient agar are added 5 c.c. of a 20 per cent solution of glucose, 10 c.c. of a 20 per cent solution of sodium sulphite (anhydrous) and 2 c.c. of liq. bismuth et ammonii cit. The medium is boiled after the addition of the bismuth solution and poured out into Petri dishes. The surface is inoculated with watery suspensions of the bacteria to be tested or directly with thin typhoid stools. The material should be well rubbed into the surface. If the medium is heavily inoculated from a growth of *coli* on agar with a platinum loop, complete suppression of growth does not occur, whereas this is achieved after lighter inoculation with a watery suspension.

This medium inhibits the growth not only of ordinary strains of *coli* but also of those that reduce sulphites. It is not possible to say precisely what is the exact chemical constitution of the compound formed between bismuth and sodium sulphite which is active in the suppression of *coli*. A white precipitate forms on the addition of sodium sulphite to liq. bismuthi but this precipitate alone does not inhibit but combined with a certain excess of sodium sulphite it does.

A fluid enrichment medium consisting of 100 c.c. bouillon, 5 c.c. of 20 per cent glucose, 2.5 c.c. of 20 per cent sodium sulphite and 2 c.c. of liq. bismuth has proved useful in the isolation of typhi from enteric stools. In the solid medium 0.5 c.c. of a 1 per cent solution of brilliant green can be added to each 100 c.c. of the medium. Strains of coli which reduced sulphites were not suppressed by brilliant green but they were inhibited by the bismuth-sulphite combination.

Liquor bismuthi et ammonii cit. in 1 in 500 to 1 in 1000 dilution alone without sulphite inhibits the growth of *Clostridium Welchii* in glucose agar.

RENAL FUNCTION: The Dilution and Concentration Tests of Renal Function, Pratt, J. H. Bost. Med. and Surg. Jour., July 29, 1926, excv, No. 5, p. 203.

The dilution and concentration tests give important information regarding kidney function. If an abnormal response is obtained the tests should be repeated once or twice after measures have been taken to remove disturbing extrarenal factors, the chief of which are dehydration of the tissues and an excess of water in the organism. The tests should be employed in combination with the other functional methods of proved value and interpreted cautiously in the light of all facts that can be gained from the complete clinical study of the case. The tests have been found of distinct value in the detection of partial dehydration and latent edema.

The author employs the following method for the test as follows:

The patient urinates at exactly 7 A.M. and is given immediately 1000 c.c. of fluid, either water alone, or part tea and part water, as he prefers. This amount of fluid is all to be taken within five or ten minutes. At 7:30 or 8 A.M. he may have rolls, toast and an egg, or any other dry food. At 8 A.M., exactly, the urine is to be voided, and again at 9 A.M. Thereafter urine is collected every two hours until 9 P.M. The urine voided between 9 P.M. and 7 A.M. the following day is collected in one portion. Relatively dry food is given at lunch and supper, usually potatoes, cheese, meat, eggs and toast. The patient is allowed to choose which of these he desires. If thirst becomes annoying a measured amount of water is given at 5 P.M. Usually, however, in the cases reported no fluid was taken until 7 P.M. or 9 P.M. If the reaction on the part of the kidneys is normal to this test a diluted urine is voided during the morning hours, and a concentrated urine during the remainder of the day.

TUBERCLE BACILLUS: A Comparison of the Schulte-Tiggs and Ziehl-Neelson Methods for Staining Acid-Fast Bacteria, Simmons, J. S., and Steves, E. J. Am. Rev. Tuberc., July, 1926, xiv, 102.

The authors conclude that both methods are of equal value.

LIVER FUNCTION: The Rose Bengal Test for Liver Function with Particular Reference to Its Use in the Therapy of Syphilis, Epstein, N. N., and Rauschkolb, J. E. Arch. Dermat. and Syph., August, 1926, xiv, No. 2, p. 122.

With the usual preparation for intravenous medication, a vein in the cubital fossa is selected and a medium sized needle inserted. Ten c.c. of blood is withdrawn and transferred to a centrifuge tube, marked No. 1, containing 0.4 c.c. of 2 per cent potassium oxalate solution made up in physiologic sodium chloride, which is inverted once gently. Rough handling may cause some hemolysis of the blood. The patient and blood specimens are kept in a subdued light to prevent hemolysis as a result of the photodynamic action of the dye.

The needle is left in situ and 10 c.c. of a 1 per cent solution of rose bengal (100 mg.) is injected slowly. When 5 c.c. has been injected, the time is noted and the injection completed. The syringe with which the injection is made is discarded and a clean syringe containing from 3 to 4 c.c. of sterile physiologic sodium chloride is attached to the needle in the vein. The needle is kept free from blood clot by frequent injections of small amounts of sodium chloride solution. At the end of one and one-half minutes, blood is withdrawn slowly, so that when two minutes have passed the syringe contains 5 c.c. of blood. The blood is then transferred to centrifuge tube No. 2, containing 0.2 c.c. of the potassium oxalate solution, and the tube is inverted once gently. The syringe is washed with physiologic sodium chloride, refilled with the saline solution and again attached to the needle in the vein. In a similar manner, an eight-minute specimen is obtained and transferred to centrifuge tube No. 3, containing 0.2 c.c. of the potassium oxalate solution.

This procedure differs from the original technic in that a four minute specimen is not taken, and that 0.2 c.c. of potassium oxalate solution, 2 per cent, made up in physiologic

sodium chloride instead of 2 c.c. of 2 per cent potassium oxalate solution is used. This has the obvious advantage of requiring less manipulation of the vein, of necessitating less laboratory work and of giving as much information.

The blood specimens Nos. 1, 2, and 3, control, two minute and eight minute, respectively, are centrifuged until the supernatant plasma is clear. The color in tubes Nos. 2 and 3 is then compared with a standard solution of the dye made up as follows:

Five c.c. of the plasma in tube No. 1 (control specimen) is added to 5 c.c. of a 0.0075 per cent solution of rose bengal, made up by adding 0.75 c.c. of a 1 per cent solution of rose bengal to 100 c.c. of water. This is thoroughly mixed, and 5 c.c. of the resultant solution is added to 15 c.c. of water. The original 5 c.c. of the 0.0075 per cent solution of rose bengal is thus diluted three times.

The plasma in tubes Nos. 2 and 3 is diluted twice by pipetting 1 c.c. of plasma and adding it to 3 c.c. of water. These dilutions are used for convenience in colorimetric determinations. The diluted plasmas are then compared with the diluted standard solution in a Hellige colorimeter. Since the plasma of tubes Nos. 2 and 3 are diluted in the same manner, the relationship between the color of each specimen will be constant.

Calculation of Results.—The method of calculation, as originally described, required that the blood volume of each patient be calculated so that the colorimetric readings of the various patients might be compared with a standard. The arbitrary standard selected was an individual with a blood volume of 7000 c.c. who had received 100 mg. of the dye, giving a resultant solution of 19.1 per cent of the standard prepared as above. The colorimetric reading of the plasma of the two minute specimen, after being corrected for dilution by the oxalate solution and for the solid elements of the blood which contained no dye, was reduced to 19.1 per cent at the expense of the blood volume. In other words, the colorimetric reading of specimen No. 2 was reduced to 19.1 per cent. The eight minute colorimetric reading is then compared with the standard (19.1 per cent) by the following fraction:

$$\frac{\text{8 minute reading}}{\text{2 minute reading}} \times 191 \text{ per cent} = 19.1 \text{ per cent.}$$

For example:

The two minute colorimetric reading is 70 and the eight minute colorimetric reading 35; then to reduce this to standard conditions calculate as follows.

$$\tfrac{35}{70} \text{ or } \tfrac{1}{2} \times 19.1 \text{ per cent} = 9.5 \text{ per cent.}$$

Since 19.1 per cent is the highest concentration of the dye possible under standard conditions, this figure is 100 per cent retention of the dye in the blood stream. Since we are interested only in how much dye the liver can excrete in eight minutes, the eight minute reading, reduced to the standard conditions will determine whether a patient has a normal or abnormal liver function.

The upper limit of normal as previously established is 11.5 per cent for the eight minute colorimetric reading. Regarding 19.1 per cent as 100 per cent retention of the dye, the upper limit of normal will be 60 per cent retention, and we will consider an eight minute reading of more than 60 per cent as abnormal and denoting impaired liver function.

This report is based on a total of 252 tests applied to various types of syphilitic patients.

Latent hepatic disease occurring in patients in the various stages of syphilis can be detected by means of the rose bengal liver function test.

The authors frequently found the rose bengal liver function test a valuable indicator as absolute indication of liver insufficiency.

Arsphenamine jaundice is constantly associated with definite retention of rose bengal in the blood stream. With clinical recovery, the liver function returns to normal. There is no permanent change in liver function following clinical recovery from an arsphenamine hepatitis.

Acute syphilitic hepatitis probably sometimes occurs during the secondary stage without physical signs pointing to liver disease.

The authors frequently found the rose bengal liver function test a valuable indicator as to the prognosis in cases of arsphenamine icterus. Moreover, they believe it reveals cases of acute syphilitic hepatitis which cannot be diagnosed in any other manner. The test is an adjunct in the treatment of syphilitic patients.

PEROXIDASE: A New Peroxidase Reaction, Sato, A., and Sekiya, S. Tohokva Jour. Exper. Med., 1926, vii, No. 5, p. 111.

Reagents:

1. Copper sulphate, 5 per cent aqueous solution.

2. Rub 0.2 gm. benzidine with a few drops of water in a mortar, add 200 c.c. of water, filter, and add 4 drops of 3 per cent hydrogen peroxide to the filtrate.

3. 1 per cent aqueous sanfranine.

Method:

To a fresh, dry blood smear add solution 1, drain, and add solution 2 for two minutes. Wash in water.

Place one drop of 0.05 per cent aqueous sanfranin on a slide and invert upon it the peroxidase-stained smear on a cover glass and examine microscopically.

The peroxidase granules are stained a deep bluish green.

CEREBROSPINAL FLUID: The Mastic-Whole Albumin Quotient as an Expression of the Albumin Proportions in the Cerebrospinal Fluid. G. Wüllenwrbber. München. med. Wchnschr., May 7, 1926, lxxiii, 772.

The mastic solution is prepared according to Jakobsthal and Kafka; it must be occasionally controlled by a preliminary test, which is done, according to Lensberg's suggestion, by adapting the alkali content of a stable 0.8 per cent salt solution to the sensitiveness of the labile mastic solution. One to two drops of normosal per 1 c.c. have usually been sufficient.

One c.c. of the mastic solution for use is put on one of the 2 test tubes of Dold's flocculation-meter together with 0.5 c.c. of fluid and 0.5 c.c. of a 0.8 per cent salt solution. One c.c. of the mastic solution for use and 1 c.c. of water are put into the other tube. The latter solution serves as a control. From the tube which contains the fluid and which shows more flocculation so much liquid is drawn into a measure tube, that both tubes are equally flocculated. The degree of flocculation is determined and expressed in c.c. or 0.001 c.c. The changes of the fluid-mastic mixture, which, for instance in paralysis, lead to flocculation, are also read in this way as flocculation degrees. The determination may be made three minutes after the preparation of the reaction, always after the same time, because the flocculation continues to increase. It expresses numerically the degree of flocculation, which in the mastic reaction is estimated after twelve to twenty-four hours.

The determination of whole albumin has been made by the author according to Mestresat's diaphanometric method. The results are very exact; they express differences of 0.01 per cent. In order to find the quotient, the flocculation degree of the mastic-fluid-mixture in c.c. is taken as numerator and the whole albumin content expressed in 0.01 per cent as denominator.

A table shows the result to the following effect:

Meningitis fluid with little flocculation of the colloid solution and relatively high whole albumin content gives a small quotient, below 1. Paralytic fluid with strong flocculation and lower whole albumin content, gives a big quotient, above 1. The highest values for meningitis were $\frac{5.5}{0.9}$ and for paralysis $\frac{1.8}{0.6}$. Between the two extremities, the numerical values lie exactly like the curves in colloid reactions. One case of cerebrospinal lues gave a transitional value, $\frac{1.2}{1.0}$ where numerator and denominator were nearly equivalent.

Besides distinguishing the different fluids of organic nervous diseases, accurately as the colloid reactions, the author's method offers several advantages. It substitutes the estimation of the whole albumin content numerical determination. The influence of the fluid on

the mastic solution is determined by an objective method and read as flocculation reaction, not approximately estimated. The reaction may be read immediately. In initial cases the origin of the slight increase of albumin, from the blood or from the central nervous system, may be diagnosed. One quotient expresses the quantitative and qualitative albumin conditions of the cerebrospinal fluid.

GLUCOSE ESTIMATION: The Estimation of Glucose in Biologic Material, Holden, H. F. Brit. Biochem. Jour., 1926, xx, 262.

The facts recorded in this paper have two main bearings on research on carbohydrates.

Firstly, they indicate the need of testing any proposed method of analysis on mixtures of pure substances as similar as possible in composition to the mixtures it is intended to analyze in the course of the research, as well as on as many individual substances as possible.

Secondly, they emphasize the fundamental lack of specificity inherent in the methods at present available for the estimation of carbohydrates. Statements, for instance, that a given method "does not estimate substances in the blood filtrate other than reducing sugars" are probably in need of experimental confirmation. Similar considerations apply to Harned's (1925) search for the "absolute value" of the concentration of glucose in blood.

The apparent absence of amino-nitrogen from his blood filtrates may have been due to the presence therein of sodium nitrate, arising from the decomposition of the mercuric nitrate, which would interfere with the Kjeldahl method of estimation.

The recently published micromethod of Benedict (1925) is interesting in its apparently greater specificity and the use of sodium bisulphite to increase the amount of cuprous oxide reduced by a given amount of glucose. Since, however, glucose is now shown to be capable of inducing coupled oxidations, an explanation of the beneficient effect of the former substance on these lines would seem to be at least as probable as one based on any power of combining with portions of the glucose molecule under the conditions obtaining during the reduction of the copper sulphate, and hence protecting them from the action of the alkali.

All conclusions concerning the nature of the reducing carbohydrate in blood or other biologic fluids based on the ratio of its polarimetric estimation to that based on copper reduction methods may be viewed with reserve.

If glucose is estimated by a copper reduction method in a fluid containing amino-acids the value obtained may be too high, even though the amino-acids present do not alone reduce the copper solution. The error may amount to 10 to 15 per cent and appears to be due to an oxidation of the amino-acids coupled to that of the glucose.

The Hagedorn and Jensen method is unaffected by the presence of most amino-acids in moderate concentration.

If the Wood-Ost and the Hagedorn and Jensen methods be applied to the concentrated filtrate from the blood of a rabbit in hypoglycemic convulsions following the injection of insulin, there is a great divergence between the amounts of glucose estimated to be present, due to unknown reducing substances. These methods agree more closely on normal blood filtrates.

OROYA FEVER: Etiology of Oroya Fever. I. Cultivation of Bartonella Bacilliformis. Noguchi, H., and Battistini, T. S. Jour. Exper. Med., June, 1926, xliii, No. 6, p. 851.

A pure culture of a microorganism resembling in morphology and pathogenic action Bartonella bacilliformis has been obtained from blood taken during life from a case of Oroya fever which ended fatally. The blood taken at Lima into citrate solution and transported to New York at refrigerator temperature yielded positive cultures twenty-eight days after its withdrawal from the patient.

The following media were employed:

1. Leptospira medium.

0.9 per cent NaCl	800 parts
Fresh rabbit serum	100 parts
2 per cent nutrient agar (P_H 7.4)	100 parts

Rabbit hemoglobin (made by taking 1 part of defibrinated blood with 3 parts of distilled water)____10-20 parts

2. Same, containing fresh tissue.

3. Leptospira medium containing glucose, maltose, inulin.

4. Same, plus fresh tissue.

5. Horse blood agar slant.

Defibrinated horse blood added to melted 2 per cent nutrient agar (P_H 7.4) to give concentration of 20 per cent.

6. Same, containing glucose, maltose, inulin.

7. Plain agar slant.

8. Plain broth.

The strain of Bartonella bacilliformis thus isolated grows well on the semisolid leptospira medium, and also on slant agar containing animal blood. The initial growth is not readily recognizable to the naked eye, but the presence of the organisms can be determined by means of the dark-field microscope and by Giemsa and Gram straining methods. No growth has been obtained on the more ordinary culture media. The organism is an obligate aerobe, is Gram-negative, and under certain cultural conditions motile. All the forms which have been described as occurring in human red corpuscles may be found in the cultures, and in addition many granular and coarsely irregular forms have been met with.

The inoculation of cultures of Bartonella bacilliformis into Macacus rhesus produces infection and gives rise to effects which differ with the mode of inoculation. The intravenous injection of the culture into young macaques induces a prolonged irregularly remittent fever. The organism can be cultivated from the blood over a long period, and it has been detected within the red corpuscles of the monkeys, reproducing the precise appearance observed in human cases of Oroya fever.

The intradermal injection of the culture into the eyebrow of young macaques gives rise to nodular formations rich in new blood vessels and showing the bacilliform organism within the endothelial cells. From the experimentally induced nodules cultures of the organism are readily recovered.

The paper contains fifteen microphotographs and two colored plates.

PREGNANCY, SERUM DIAGNOSIS: The Ninhydrin Flocculation Test, a New Reaction in the Blood Serum for Determination of Pregnancy, Vogel, W. Zentralbl. f. Gynäk., June 12, 1926, 1, 1554.

Place 1.75 c.c. of blood serum, 10 c.c. of water (see below) and 0.2 c.c. of 1 per cent ninhydrin solution in a test tube and boil three minutes in a water bath.

The albumin of nonpregnant serum forms coarse flakes which readily separate from the bluish opaque fluid. In serum from pregnant women the albumin either remains in solution or, if the pregnancy is early, fine, bluish flaky precipitation occurs.

Upon standing, in positive reactions a light, finely flaked, grayish blue or blue precipitate covers the bottom of the tube and is readily resuspended on shaking.

With a negative reaction the flakes are coarse, fill about one-third of the tube, and frequently turn a reddish-violet.

It is necessary to use a specially prepared "standard" water: Two and five tenths grams of bicarbonate of potassium are dissolved in 945 c.c. of distilled water to which are added 55 c.c. of official lime water solution. The air is forced from a 10 liter flask by admission of carbonic acid from an ordinary carbonic acid bomb tube with the pressure regulating valve.

The solution obtained is put into the flask filled with carbonic acid gas, the opening closed with the palm of the hand, and the flask is shaken until the hand is no longer sucked into the opening. The finished solution is kept in seltzer water containers with patent stoppers.

Such a standard solution is stable only for 6 to 8 days and is useless when it becomes transparent during the test.

The blood must be taken from the fasting patient.

Five hundred cases were examined, divided into three groups:

I. Cases of existing or recent pregnancy (97.8 per cent postive reactions).

II. Nonpregnant, (97.7 per cent negative reactions).

III. Miscellaneous: A positive reaction is obtained with the serum of children under one year; cord blood, however, was negative.

Where pregnancy is less than three months the reaction fails.

In fifteen cases of amennorhea the test was correct in all but two (six of the eight positive reactions were corroborated later evidence of pregnancy).

CANCER: The Significance of Blood Coagulation Valency, Boch, H., and Rausche, C. Zentralbl. f. Chir., June 5, 1926, liii, 1440.

The method followed by the author as a test for cancer is as follows:

A 2 per cent solution of magnesium sulphate is poured into ten reaction glasses in amounts increasing from 1 to 10 drops. One c.c. of blood is put into each glass, after removal from a vein by means of a Record syringe. This mixture is shaken and let stand for two hours. The glasses are then examined to determine which amount of the solution has been able to prevent the coagulation of 1 c.c. of blood. Normally, 3 to 5 drops are sufficient, and this amount is nearly constant for each person, under varying conditions of temperature, diet, etc.

In eighteen cases examined by this method and later proved by operation to be cancer patients, sixteen or 88 per cent, showed an increase in coagulation valence to from 7 to 11 drops of the solution. Of twelve cases of ulcus ventriculi, all showed a decrease to from 1 to 3 drops. This appears to be pathognomonic.

In forty cases of various diseases such as neurosis, tuberculosis without fever, nephritis, gall bladder affections, diabetes, etc., thirty-five cases showed a normalcy of from 3 to 6 drops. Three cases of icterus showed a decrease of from 1 to 3 drops. The two others (diabetes and cholecystitis) produced an increase to seven drops. A second examination lowered the second case to 5 drops.

The method is proposed as an aid to the diagnosis of cancer.

DIPHTHERIA STAIN: New Stain for Diphtheria Bacilli, Von Staltenberg, H. Deutsch. med. Wchnschr., July, 1926, lii, 1304.

Formula:

0.250 gm. malachite green.
0.050 gm. toluidin blue.
0.010 gm. hematoxylin or logwood extract.
100 cc. of water.
3 c.c. acetic acid.
3 c.c. ethyl alcohol.

The solution is used for a minute; the specimens are then washed with tap water and dried. The granules are stained red and the center green.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Textbook of Bacteriology**

THERE are numerous bacteriologies on the market with the best of which Dr. Ford's new volume may well challenge comparison. In it he has attempted, with unqualified success, to describe completely the bacteria commonly encountered in medicine, comparative pathology, and hygiene and public health, the descriptions being in large measure based upon his own observations over a number of years in McGill and Johns Hopkins Universities and the Rockefeller Institute.

The first section of the book (seven chapters, 171 pages) is devoted to an excellently presented exposition of various technical procedures.

Section II (607 pages) is devoted to systematic bacteriology concerned especially with bacteria responsible for disease in man and animals. This section is exceedingly well done, its contents are readily accessible and of immediate practical value, and it is one of the most easily utilizable the reviewer has encountered.

In Section III (38 pages) the distribution of bacteria is discussed, followed by 96 pages devoted to infection and immunity in Section IV, also a very excellent presentation.

The fifth section (68 pages) is devoted to spirochetes, and the last section (54 pages) to infectious agents of undetermined character.

The illustrations, free-hand drawings, are excellent in every way and are truly illustrative without being diagrammatic.

The book can be recommended without reserve as an authoritative, comprehensive, and utilizable text.

Recent Advances in Hematology†

AS STATED by the author in his preface, "the production of an adequate book dealing with recent advances in hematology would require an almost encyclopedic knowledge of the vast literature of the subject," but while the present volume is small and compact, it represents an admirable survey of a complicated and most important subject.

Cytologic blood examinations, with which this book is entirely concerned, are, perhaps, one of the most frequently applied of clinical laboratory procedures.

Like all laboratory methods of examination they achieve their greatest clinical value when properly interpreted, and to this end the matter clearly and excellently presented by Dr. Piney brings much that is not only of interest but of marked clinical value.

The first two chapters are devoted to a consideration of the reticuloendothelial system and the development of blood cells.

Piney says: "The fact that the formed elements of the blood are not developed in the circulation, but are derived from the activities of parent cells in various organs makes clear the error of the conception of the blood as a special form of connective tissue with a fluid matrix; it is really in the nature of a mixed secretion of a variety of origins."

The review of the reticuloendothelial system is an excellent exposition of a disputed subject.

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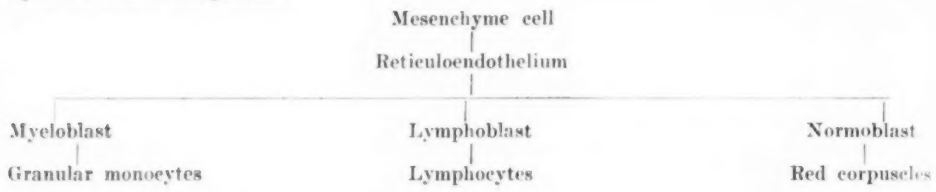
*Textbook of Bacteriology. By William W. Ford, M.D., Professor of Bacteriology, School of Hygiene and Public Health; Lecturer on Hygiene, School of Medicine, Johns Hopkins University. Octavo of 1069 pages with 186 illustrations. Cloth. W. B. Saunders Co., Philadelphia and London, 1927.

†Recent Advances in Hematology. By A. Piney, Research Pathologist Cancer Hospital, London. Cloth. Pp. 276. 4 colored plates and 18 text illustrations. P. Blakiston's Sons and Co., Philadelphia.

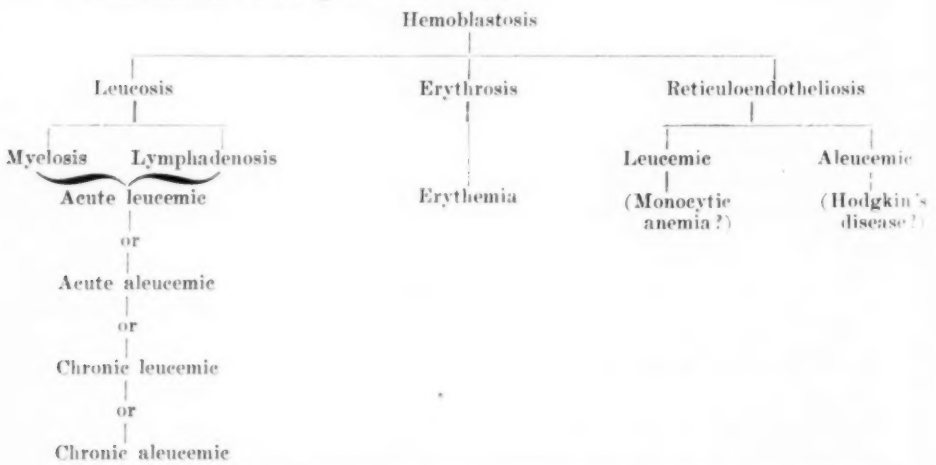
NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

The author subscribes to the following view as to the process of blood formation based upon his own investigations:



The classification following is also of interest:



Chapters V and VI devoted to leucemoid blood-pictures (infective and noninfective) are of great practical value and should be read by every practitioner as also should the chapter on symptomatic blood changes.

The appendix describes in detail the hematologic technic comprising the author's concept of a complete blood examination.

The typography and the illustrations are excellent.

This book is one which the practitioner should buy, not borrow, as he will find it a valuable desk companion.

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*A Textbook of Pathology**

TIME and trial are stern censors, and when a textbook achieves an eighth edition, one may rest assured that it is of value to its readers.

As the authors state in their preface "details of morbid anatomy and physiology, and to a reasonably sufficient extent, of etiology, can and should be gathered in a convenient form for students."

This eighth edition of Stengel's *Pathology*, prepared in collaboration with Dr. Herbert Fox, is truly a new edition and not merely a reprint. The revision has been extensive, comprehensive, and thorough, and the text may be accepted as an authoritative statement of present day concepts.

Intended primarily for students, the style is clear and graphic and one of easy fluency. If at times a trifle dogmatic, for the student this is an advantage, and for the more experienced reader it is not a defect, for the main purpose of medical texts, after all, is to stimulate thought and collateral reading.

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*A Textbook of Pathology. By Alfred Stengel, M.D., Sc.D., Professor of Medicine, University of Pennsylvania, and Herbert Fox, M.D., Professor of Comparative Pathology, and Director of The Pepper Laboratory of Clinical Medicine, University of Pennsylvania. Eighth Edition, Reset. Octavo. Pp. 1138 with 552 text illustrations, many in colors, and 18 colored plates. Cloth. W. B. Saunders Co., Philadelphia and London.

The typography and illustrations, which are well chosen and reproduced, constitute an excellent example of the printer's art.

The volume can be unhesitatingly recommended to the student and practitioner and will be of use in the laboratory as a reference volume.

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*Differential Diagnosis of Internal Medicine**

HEALTH and disease may be looked upon as opposite extremes of functional efficiency, health being regarded as a condition dependent upon perfect performance of function, and disease a condition characterized by disturbance or loss of function.

Diagnosis, the first essential step in the management of disease, rests upon the determination, first, of the particular function or functions affected; second, of the degree of impairment; third, whenever possible, upon the detection of the responsible cause and its mechanism.

In the general practice of medicine the presenting, the obvious, or the troublesome symptom is, as a rule, the direct cause of the visit to the physician, and unless carefully considered and correlated with other findings may often overshadow the more important underlying cause.

It is as an aid in such correlation, as a stimulus to further clinical studies, and as an indicator of diagnostic possibilities that such volumes as that of Professor Mathes find their true use.

In this, the translation of this well known German text, the translators have included American and English methods applicable to the differential diagnosis of disease and have made such other additions as were required to bring the work up to date.

The twenty-four chapters of this work include a consideration of the differential diagnosis of acute febrile conditions; diseases characterized by moderate fever, the meningeal and peritonitic symptom complexes, ileus and intestinal stenoses; diseases of the larynx and trachea; diseases of the lungs and pleura; circulatory diseases; the spleen, liver, and biliary ducts; the gastrointestinal tract; the pancreas; the urinary organs; the blood; endocrine glands; diseases of bone; neuralgia-like pains, and headache.

The discussion under these headings is clearly written in an eminently practical manner which emphasizes the practical experience of both the author and the translators.

Diagnosis is an art to be learned by studies of the patient and cannot be acquired from books alone. Books, however, are valuable aids in such studies as crystallizing the impressions formed, and there are few volumes of this type that will prove more generally useful to the physician at large, to whose library Professor Mathes' book will be a valuable addition.

Typographically the volume is an excellent example of the printer's art.

City Health Administration†

THIS is a very good outline of the principal functions involved in municipal health administration, of which the author has made a study, of interest not only to the medical man and the sanitarian, but in which he has also tried to keep the layman's viewpoint in mind. The author has approached the subject matter from the viewpoint of a student of municipal government as a whole, and it is not surprising that his recommendations embody a rather close association or amalgamation between public welfare and public health. An exception to this viewpoint may be taken by the health administrator who has been closely identified with the endeavor on the part of progressive health departments to unload the driftwood of charity and welfare and other activities having only an indirect influence upon prevalence of disease.

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*A Textbook of the Differential Diagnosis of Internal Medicine. By M. Mathes, M.D., Professor of Medicine University of Königsberg. Authorized translation of the fourth German edition by Drs. I. W. Held and M. H. Gross. Pp. 908. 176 Illustrations. Cloth. P. Blakiston's Sons and Co., Philadelphia.

†City Health Administration. By Carl E. McCombs, M.D., National Institute of Public Administration and New York Bureau of Municipal Research. Pp. 524. Cloth. The Macmillan Company, New York, 1927.

Dr. McCombs has not drawn to any great extent upon the recommendations which have emanated from the various Committees of the American Public Health Association, and more especially, the Committee which has been responsible for the formulation of the Appraisal Form for City Health Work. On the other hand he shows that he has a good acquaintance with the purposes of the Association and its ideas and ideals which are representative of the best thought among the American health administrators.

His book is divided into three parts, the first dealing with general municipal health functions. It is the second part which will prove of the greatest interest to the sanitarian, and this section constitutes by far the greatest portion of the book. The author discusses the organization and administration of sickness preventive functions, taking up in proper sequence the organization of a board of health, a description of the duties and responsibilities of the health officer and his employees, an outline of a vital statistical service and bureaus for the prevention and control of disease, promotion of child hygiene and public health nursing, food inspection, sanitary inspection, laboratory service, and public health education. The third portion of the book is devoted to a discussion of the organization and administration of sickness treatment functions with chapters on hospital management, medical and nursing services of hospitals, record keeping, etc.

The book is written in an orderly fashion, and each discussion leads most appropriately to the paragraphs that follow. The general outline is admirable, although it must be admitted that in some of the detailed recommendations there could not be complete approval on the part of those who have had many years of practical experience in municipal health administration. The health administrator has waited for many years for a good outline on administrative procedure, and it is the opinion of the reviewer that to date, at least, the present volume is the best and most modern contribution which has come to his attention.

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*Ergebnisse der medizinischen Strahlenforschung (Roentgendiagnostik, Roentgen-, Radium-, und Lichttherapie)**

THIS is the first of a series of volumes containing monographs written by those eminent in various fields of radiology. Although the number of volumes to be issued is not stated, it is intended that both clinically and theoretically all phases of radiology will be adequately covered. There are ten monographs in this volume which consists of approximately 700 pages, printed on good quality paper with adequate illustrations of high quality. Abundant clinical data is given to support the conclusions drawn, and the methods employed are fully explained. The monographs of clinical interest include *Inflammation and Malignant Conditions of the Large Intestine* by Fischer; *Bone Atrophy* by Friedl and Schinz; *Acute Miliary Tuberculosis* by Lorey; *The Treatment of Carcinoma of the Cervix* by Lahm; and *X-Ray and Radium Treatment of Carcinoma of the Esophagus* by Kurtzahn. The general and theoretical subjects treated are *The Spectroscope in Medical Roentgenology* by Grebe; *The Ionization Measurement of X-ray* by Kustner; *The Effect of X-ray on the Testicle* by Schinz and Slotopolsky; *Physical Sensitization* by Holthuse and *Protection Against X-ray and Plans for a Roentgen Division of a Hospital* by Glocker.

In his *Treatment of Carcinoma of the Cervix* Dr. Lahm reviews the end-results of operation in carcinoma of the cervix, and gives an extensive comparison of treatment by operation and by radiation. Five years freedom from recurrence is considered a cure, although it is recognized that in rare instances recurrences occur after ten to fifteen years. The results of Döderlein and Kroenig are among those extensively quoted. In general, approximately 20 per cent of the cases remain symptom-free for five years when treated surgically, and the same percentage, when treated by radiation. Radiation of inoperable cancer gave approximately 10 per cent cure in a series of 2,000 cases from seventeen different clinics. The author concludes that operation and radiation when in competent hands are of approximately equal value.

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*Ergebnisse der medizinischen Strahlenforschung (Röntgendiagnostik, Röntgen-, Radium- und Lichttherapie). Edited by H. Holfelder, Frankfurt a.M., H. Holthusen, Hamburg, O. Jungling, Tübigen, H. Martius, Bonn a.Rh. Vol. I. Published by Verlag von Georg Thieme, Leipzig, 1925.

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EDITORIALS

The Cardiac Minute Output or the Velocity of Blood Circulation

WITHIN the last year considerable interest has been manifested in the velocity of blood flow, the circulatory minute volume in the peripheral arteries, and also in the pulmonary and the coronary arteries; the cardiac output, the pulse wave velocity and the capillary bed reaction, all under normal and abnormal conditions. Many ingenious methods have been evolved for the determination of each of these rates and the establishment of some recent conceptions of the mechanism of the circulation and some of the controlling factors. The volume of carbon dioxide produced to the volume of oxygen consumed and the difference between the arterial and the venous oxygen tensions are indices of the cardiorespiratory function. The newer methods for obtaining these facts in man are most promising. The results have already led to rather striking conclusions.

The original method of Fick has been much elaborated and perfected by Burwell and Robinson (I*) and has been extensively employed by them with human subjects and by Harrison, Leonard, and Blalock with dogs in the

*The roman numerals indicate the number of the respective study in the series of studies by the group of authors whose work is being discussed.

accumulation of much interesting data. Robinson and his coworkers consider the evidence secured as satisfactorily establishing the facts that (II) the output of the heart is greatly increased when heart failure sets in and reduction of the output of the heart is favorable to the reestablishment of adequate functional circulatory efficiency. (III.) Digitalization, it was demonstrated in animals and later in man, produces, contrary to expectation, a fall in cardiac output.

This group of workers has by this method further demonstrated that (IV) the cardiac output in pneumonia is usually increased, depending not on changes in oxygen consumption, but on the accompanying anoxemia and the anemia. The strain on the myocardium is thus increased. (V.) Diminution of the hemoglobin content of the blood is associated with an increased cardiac output. Progressive hemorrhage, on the other hand, leading to decreased blood volume, causes no marked change in the cardiac output until the animal approaches a state of shock when the cardiac output rapidly decreases. With the maintenance of the blood volume by saline, with, nevertheless, an unavoidable diminution of the hemoglobin by dilution, there is an increased output which diminishes with shock. (VI.) Anoxemia of severe degree (30 per cent unsaturation) causes increases up to 500 per cent in the minute cardiac output.

Besides the effects of digitalis (I) on the cardiac output of dogs, Pilcher, Wilson, and Harrison have studied the effects of barium, calcium and potassium (II), caffeine sodium benzoate (III), and epinephrin (IV) on the circulatory minute volume in dogs. In these studies, some unexpected results were again obtained. For instance, caffeine sodium benzoate in full therapeutic doses showed only slight, but definite, decrease in the minute ventricular ejection. Epinephrin, however, as was to be expected, produced, as an immediate effect, an increase in the output of the normal dog's heart. Thus, the treatment of "shock" or hemorrhage which had previously been proved to decrease the cardiac output was, according to the authors, rationalized.

It is fortunate indeed that paralleling these studies of the Nashville group, there are, in almost equal number, studies of similar phenomena by a new and altogether different means of approach.

Blumgart, Weiss, and Yens have perfected (I) an active radium deposit intravenous injection method for the determination of the velocity of blood flow. The estimation of the circulation time from the injection into one arm to the appearance of the radium in the opposite arm shows less than a three second variation. (II.) The average arm to arm circulation time for normal individuals of all ages was eighteen seconds, when reduced to square meter of body surface it was ten seconds at the ages of fifteen to twenty-nine, twenty seconds between thirty and seventy-five years. The velocity of blood flow is more rapid in children. Increased pulse rate increases the velocity of blood flow in one and the same individual. Normal low ventricular rate, however, does not lower the velocity below that for other normal individuals with higher rates. Normal variations in blood pressure have apparently no great effect on velocity of blood flow. (III.) In rheumatic and syphilitic heart disease (A) without valvular damage, three patients with cardiac over-

activity of rheumatic origin showed normal or slightly increased velocity. Three with signs and symptoms of severe myocardial damage showed slightly prolonged velocity. (B.) With valvular lesions and regular rhythm, eight studies showed circulation times within normal limits in the absence of cardiac decompensation and with slight or great prolongation according to the degree of heart failure. The venous pressures were normal. The vital capacities were reduced. The dysfunction, thus, is seemingly due to myocardial damage. The site and extent of the valvular lesion seemed to play no part. (C.) With valvular disease and fibrillation of the auricles (1) the circulation time was twenty-nine to fifty-five seconds, depending upon the circulatory compensation.

In syphilitic heart disease with aortic valve lesions, the circulatory velocity was prolonged only where there was evidence of circulatory failure and in degree corresponding with cardiac pain and aortic dyspnea, and with no signs of congestive failure normal velocity of blood flow may be present. This suggests that these symptoms are not necessarily due to congestion of the pulmonary vessels, but may be due to a reflex mechanism. In 53 normal individuals the range of blood flow velocity was from eleven to twenty-four seconds, averaging eighteen seconds, while in 86 cardiovascular cases it was from eleven to seventy-three seconds, averaging thirty-three seconds.

(IV.) In arterioselerotic cases without symptoms and signs of cardiac failure, the average was twenty-four seconds, while in the same type of case with symptoms and signs of cardiac failure the average was thirty-eight seconds. Thus, a slight retardation in velocity occurs in general before symptoms and signs of cardiac failure become manifest. The degree of retardation corresponds to the degree of cardiac failure.

In *hypertension* cases *prolongation* of the velocity of blood flow was observed without the presence of any evidence of circulatory embarrassment. Auricular fibrillation, complicating any case, induces a disproportionate prolongation over those with regular mechanism and cardiac failure. Vital capacity reduction appears first, then velocity retardation, then venous pressure increase. With recovery from failure venous pressure first returns to normal, then velocity of blood flow, and lastly vital capacity.

Retardation of velocity appears before symptoms and signs of failure and returns toward normal before there is clinical evidence of improvement.

(V.) The physiologic and pathologic significance of the findings in regard to velocity of blood flow are reiterated. The path traversed by radium *C* is considered proved to be uniform from patient to patient. Excluding all possible local causes, the authors have found that the rise in venous pressure is preceded by a definite period, when the vital capacity is reduced and the velocity of the blood flow is lessened. A study of the anatomic and physiologic characteristics of the veins is thought to afford the explanation of this precedence.

The authors then elaborated their radium active deposit method so as to detect the ionization current produced by the gamma rays at the moment that the radium active deposit reaches the right heart as well as detection of the time of arrival in the arteries about the elbow. The difference between

these times after the application of a standard correction gives a measurement of the velocity of blood flow through the lungs. In 50 normal individuals, the pulmonary circulation time ranged from four and a half to seventeen seconds, with an average of eleven seconds. In cardiac failure the pulmonary circulation time was sixty-eight seconds, while the venous circulation time was thirty-four seconds. In emphysema, the velocity of blood flow through the lungs was found to be normal. Digitalization of normal individuals produced no demonstrable change in the velocity of pulmonary blood flow, whereas, in cardiac patients in whom there was definite clinical improvement following digitalization, the velocity of blood flow through the lungs was increased.

This finding of increased velocity of pulmonary blood flow following digitalization apparently coincides with the clinical conception of the effect of digitalis and seemingly disagrees with the unexpected conclusions of Robinson, Burwell, and their coworkers. It may be, however, that the effect of digitalization may be distinctly different on the pulmonary circulation as contrasted with the systemic circulation. Vascular changes resulting in pressure changes have been shown by both methods to produce changes in the cardiac output, and it may possibly be that the output of one or the other ventricle can be independently affected.

Brocklehurst, Haggard, and Henderson estimated that about 100 c.c. of blood is pumped on through the lungs and on to the tissues for each 4 c.c. of oxygen consumed at rest. The circulation efficiency is so great that the arteriovenous carbon dioxide difference is on the average only about 3.5 per cent by volume and the oxygen difference only slightly above 4 per cent by volume. An oxygen consumption of 240 c.c. per minute requires a circulation of 6,000 c.c. of blood. The normal circulation is thus such that with the blood giving up only 4 per cent by volume in passing through the capillaries, the tissues are kept in a pressure of oxygen. The efficiency of the circulation depends on its capacity to maintain these conditions. The measureable factor of this limitation of power is the relation of the oxygen content of the arteries to that of the venous blood. With inefficiency, the difference increases. This is what is termed an increased oxygen debt.

Henderson, Haggard, and Dolley have studied men of various grades of activity and observed that in passing from rest to exercise, the nonathlete approximately doubles his circulation, while the athlete triples his blood flow. In moderate exertion, the adjustment is proportionate to the oxygen consumption and the energy expenditure. The average normal stroke volume at rest is about 1.5 to 1.8 c.c. per kilogram of body weight per beat. In the athlete, the pulse rate tends to be slower and the stroke volume distinctly greater at rest as well as on exertion by which it may be increased 50 per cent. Increasing pulse rate decreases the stroke volume. The slow pulse allows longer diastoles for better relaxation and filling of the ventricles.

Halsey, Reynolds, and Blackberg, of Tulane University, using a slightly modified technic of Marshall, made some important observations. These investigators found the cardiac output in the dog usually *increased* by chloral hydrate, quinine, and quinidine, and decreased by chloroform, homocamfin

(cyclosal) and ephedrin alone or after chloral and chloroform. The accompanying heart rate, blood pressure, and oxygen consumption rate changes were recorded and are carefully discussed, as are, also, the possible explanations of the findings. More of this type of fundamental and laborious pharmacologic research will guide clear and rational conceptions of the effects of drugs.

However, there is as yet not sufficient corroborated data obtained by various methods and various groups of workers to justify sweeping conclusions or to substantiate new theories. New and interesting light has been shed on these phenomena. The improved ethyl iodide method of Henderson and Haggard, an improved method which J. W. Moore and his associates are working with, and the promise of an improved method from E. K. Marshall, all together hold forth hope that the next few years will yield much corroborated data on the subject of the velocity of blood circulation and the minute cardiac output under normal physiologic, pharmacologic, and pathologic conditions.

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—*G. R. H.*

The Advantage of Early Examination of Diseased Tissue

WHEN a patient presents himself with a lesion of the skin, particularly of the face, which is suspected of being cancerous, the most important point to determine is the type of the lesion. Owing to propaganda and the general education of the public with regard to cancer, physicians generally are seeing malignant lesions earlier than formerly. A fairly certain method of determining the type of lesion is either by biopsy or by excision of the diseased area and its examination with a microscope. The local lesion itself is not the most important consideration, for by known therapeutic agents (excision, electric coagulation, or irradiation) this area can usually be controlled. Of much more importance is the determination of the type and grade of malignancy of the original tumor, if it is a neoplasm, so that intelligent procedures regarding the adjacent lymph nodes can be planned and executed.

Certain observers have assumed that biopsy is dangerous because of the risk of causing more rapid spread of the growth. The evidence usually offered was the attending physician's impression that growth is more rapid following removal of tissue. On the other hand, experimental evidence has accumulated in carefully controlled observations to support the opinion that biopsy of a malignant tumor has no apparent influence on its growth or spread.*

It seems more logical to run the small risk, if there is such, in biopsy for the purpose of examining excised tissue and establishing a definite diagnosis, rather than inaugurate treatment for a lesion whose exact nature has not been determined.

It frequently happens that an early lesion of the skin is treated and destroyed, and the site of the affection heals without the exact pathologic change having been determined. Later adjacent lymph nodes may enlarge and subsequent examination of tissue reveal carcinoma. If the original lesion or a part of it had been examined microscopically, the type and grade of malignancy determined, and appropriate treatment instituted, such as the removal of adjacent lymph nodes, the patient might have been given a better prognosis.

If chronic lesions develop in affections of the skin, particularly of the face, in which malignancy must be considered in the differential diagnosis, much valuable time is saved if a part of the diseased area which is a fair sample of the whole, or the entire lesion, is excised and examined by a pathologist.

—*H. D. C.*

*Wood, F. C.: The Experimental Pathology of Cancer, Jour. Am. Med. Assn. 1925, lxxxiv, 4-8.